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This report summarizes progress for the first year of the subcontract, AFOSR F49620-91-C-0063 entitled "Effects of Halogenated Hydrocarbons on aquatic organisms".

This research dealt with several experiments evaluating the response of different algal species towards selected halogenated hydrocarbons. Two groups of algal species were assayed. The response of the algal species towards the chemical was evaluated under various growth medium composition. With respect to changes in the growth medium composition it was clear in this work that depletion of nutrients nitrogen or phosphate in case of green algal species or silicate in the case of diatoms lowers the percentage of survival of the organism. Green algal species were more or less tolerant to changing growth media composition than diatoms. In conclusion, when bioassaying the halogenated hydrocarbons, various algal species as well as growth medium composition should be considered.

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**Effects of Halogenated Hydrocarbons on Aquatic Organisms**

**First Annual Technical Report**

**to**

**Air Force Office of Scientific Research**

**Contract AFOSR: F49620-91-C-0063**

**August 30, 1993**

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## SUMMARY

This report summarizes progress for the first year of the subcontract, AFOSR F49620-91-C-0063 entitled "Effects of Halogenated Hydrocarbons on aquatic organisms".

This research dealt with several experiments evaluating the reponse of different algal species towards selected halogenated hydrocarbons. Two groups of algal species were assayed. The response of the algal species towards the chemical was evaluated under various growth medium composition. With respect to changes in the growth medium composition it was clear in this work that depletion of nutrients nitrogen or phosphate in case of green algal species or silicate in the case of diatoms lowers the percentage of survival of the organism. Green algal species were more or less tolerant to changing growth media composition than diatoms. In conclusion, when bioassaying the halogenated hydrocarbons, various algal species as well as growth medium composition should be considered.

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## INTRODUCTION

This report summarizes progress for the second year of the subcontract, AFOSR F49620-91-C-0063 entitled "Effects of Halogenated Hydrocarbons on aquatic organisms.

Chlorinated hydrocarbons are natural components of oil deposits and commonly find their way into surface waters as a result of discharges from refineries, waste oil, disposal, and accidental spills. Municipal wastewater discharges have also been recognized as sources of aliphatic and aromatic hydrocarbons (Barrick, 1982). Chlorinated hydrocarbons may enter the environment as a result of their use as solvents, heat transfer fluids, flame retardants or chemical intermediates or as waste products of the elector-industry (Jan and Malnersic, 1980). Among the most common solvents used form halogenated hydrocarbons are: trichloroethylene, tetrachloroethylene and dichloroethlene. These compounds are among the dominant contaminants detected in gound water (Barber et. al., 1988: Love and Eilers, 1982). Organic solvents can make their way into the environment as industrial wastes. Because of their carcinogenic potential, contamination of soil and water by solvents is cause for *serious concern*.

Relatively few reports have been published on the comparative toxicity of solvents towards tests organisms, and these dealt primarily with fish and aquatic invertebrates (Alexander et. al., 1878: Bouman et. al., 1981: LeBlanc and Suprenant, 1983). However, only few data of toxicity effects of solvents on algae have published (pearson and McConnell, 1975; Lay et. al., 1984; Stratton, 1987).

Algae have been considered to be good indicators of bioactivity of industrial wastes (walsh et. al., 1984). Algae are ubiquitous in aquatic ecosystems, where they incorporate solar energy into biomass, produce oxygen that is dissolved in water and used by aquatic organisms, function in cycling and mineralization of chemical elements, and serve as food for herbivorous and omnivorous animals. When they die, they sink as food for herbivoruous and amnivorous animals. When they die, they sink to the sediment where their chemical constituents are transformed, solubilized, and recycled into the water. These functions are dependent upon phytoplankton population dynamics which, in turn, depend

upon seasonal variability in temperature, intensity of solar radiation, nutrient concentrations in the water, and grazing by animals. Natural and anthropogenic alterations of water, and grazing by animals. Natural and anthropogenic alterations of water quality can upset the balance of these controlling factors and bring about changes in species composition of the algal community, rates of production, biomass, and water chemistry. If water quality is altered by toxicants or growth stimulants from industrial, agricultural or municipal sources, normal algal function may be upset, causing gross changes in structure and function of the receiving aquatic ecosystem.

#### **OBJECTIVE:**

During the second year of the project the following studies were performed in order to:

.Compare the response of fresh water and saltwater (estuarine) single algal species, to different concentrations of the halogenated hydrocarbons, under different growth parameters: nutrients and salinities

#### **EXPERIMENTAL DESIGN:**

The response of algal species to chemicals was determined at 20 ° C and 30 ° C, under two light irradiances : 80 and 120  $\mu\text{Em}^{-2} \text{ s}^{-1}$

#### **MATERIALS AND METHODS:**

##### **Algal species:**

Assays were conducted with freshwater and saltwater algal species:

##### **Fresh water Green :**

*Gleocystis sp.*, *Tetraselmis sp.*, *Chlorella sp.*,  
*Nannochloris 3 sp.*, *Selenastrum capricornutum sp.*,  
*Nannochloris sp.*, *Scenedesmus basilensis sp.*, and *Chlorococcus sp*

##### **Salt water (estuarine): Diatom :**

*Cyclotella*, *Nitzschia pusilla*, *Navicula saprophila*, *Nitzschia dissipata*,  
*Thalassiosira weissflogii*, *Skeletonema*, *Amphiprora hyalina*, *Thalassiosira*

*pseudonana*, *Cyclindrotheca*, *Cyclindrotheca*, *Chaetoceros muelleri*, and *Minutocellus* sp.

All algal species were obtained from the University of Texas algal collection (UTEX). The algal species were checked for bacterial contamination before use.

**Culture Medium:**

"F/2" Guillard and Ryther (1962)

**Macroelements:** (concentration mM/L medium)

NaNO <sub>3</sub> 0.88	NaH <sub>2</sub> PO <sub>4</sub> 0.036	Na <sub>2</sub> SiO <sub>3</sub> 0.107
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**Trace Metals** (concentration uM/L medium):

Zinc	0.08	Copper	0.04
Manganese	0.90	Iron	11.70
Cobalt	0.05	EDTA	11.50
Molybdenum	0.03		

**Vitamins:** (concentration ug/L medium)

Cyanocobalmin	0.05
Biotin	0.05
Thiamine, HCL	100.00

The culture medium was used for all species. For marine species, the medium was enriched with commercial artificial sea salt mix (Instant ocean, aquarium system, Inc. East Lake, OH.) to 20 parts per thousand (ppt) salinity.

Distilled water was used for preparation of media. The pH of media was adjusted to 8.0 with sodium hydroxide.

**Inoculum:**

Inoculations were prepared with cultures in log growth phase, obtained by frequent replenishment of medium. Cultures were acclimated to the growth conditions of the treatment for 72 h prior to the exposure by maintaining the growth rates constant. The initial inoculum was standardized to  $7 \times 10^4$  cells/ml. in all treatments.

**Culturing:**

All cultures were performed in triplicate in sterile optically matched tubes. Cultures were incubated on shakers in incubators at one temperature (30° C)

under one light irradiation ( $80 \text{ uE m}^{-2} \text{ s}^{-1}$ ), in light-dark cycle (16hr.light: 8hr. dark).

#### **Chemicals for tesing:**

The following volatile halocarbons were tested:

Carbon Tetrachloride, Chloroform, Trichloroethylene and Tetrachloroethylene. Test compounds were ordered from J.T. Baker Chemical Co.

#### **Concentrations and Treatments:**

All test organisms were assayed in water-solubale fraction concentrations of 0.05, 0.1, 0.2, 0.3. The 100 % solution was prepared by adding part of chemicals to 100 parts dilution water (volume to volume) and stirring in a covered glass bottles with Teflon-coating-lined screw caps for 2 hours. After allowing the solution to settle for 1h, the water-soluble fraction was siphoned into another container for distribution to the test containers. The assay was carried out in tubes containing 25ml medium. All assays were conducted in triplicate test tubes.

All algal cultures were treated with different concentartions of the halocarbon. The concentration of halocarbons was not measured, because the gas liquid chromatograph was not yet operated.

#### **Growth Monitoring:**

Cultures were incubated for 96 h. The population density was determined by cell counting using a hemacytometer. Ten microscopic fields were counted and averaged. Responses of species were estimated by:

- A. Population density measured by cell counting using Hemacytometer. From population density the growth rate ( $u$ ) of each species was calculated from the expression:

$$u = \frac{\log 10 N - \log 10 N_0}{t - t_0}$$

Where:

$N$  = population density at the end at the test

$N_0$  = population density at the beginning of the test

$t - t_0$  = length of time of the test

- B. Toxicity was calculated in percentages of the control



## **QUALITY CONTROL AND STATISTICS :**

Culturing media were sterilized by autoclaving before treatment with hydrocarbons. All glass used for experiments were also sterilized by autoclaving. The temperatures of autoclaves were monitored on a per-use basis. Spectrophotometers, pH meters, and analytical balances were calibrated on a regular basis. All glassware (pyrex) were cleaned using 1% HCL followed by rinsing thoroughly with deionized water. The triplicate tests analyzed at each parameter (e.g. Temperature, salinity...) each test was performed twice. All errors were expressed as the standard error of the mean (SEM) Occupational safety and Health Administration (OSHA) regulations regarding the safe handling of chemicals and safety of personnel were followed.

## **RESULTS AND DISCUSSION :**

The chemicals were tested, after being dissolved in acetone as we proposed in the proposal. We find that acetone, alone stimulates the growth more than the control. Therefore the chemicals were dissolved in water at very low concentration (see methods).

### **Response of species to solvents under different growth conditions.**

#### **I. Growth Media Composition:**

##### **A. Green species:**

##### **1. *Gleocystis sp.* (Figure 1)**

The response of the alga was more or less the same to all chemicals in all media except trichloroethylene was somewhat affected by the deficiency of P or N, when compared with its effect in complete medium. The survival rate of the alga was higher in complete media than media depleted in N or P.

##### **2. *Tetraselmis sp.* (Figure 2)**

The survival rate of the alga was less in the media deficient in P or N than in the complete medium especially in tests treated with 0.1% solvents

**3. *Chlorella sp.* (Figure 3)**

The survival rate of the alga was not affected by changing the composition of the medium because the alga was sensitive to the solvents when tested in complete medium

**4. *Nannochloris 3* (Figure 4)**

The alga was sensitive to changes in the nutrients of the medium at concentration 0.05% Increasing the concentration of the solvents lowered the survival percentage of the alga

**5. *Nannochloris sp.* (Figure 5)**

The survival rate of the alga was sensitive to changes in the nutrients of the medium at concentration 0.15%

**6. *Scenedesmus sp.* (Figure 6)**

The survival rate was more affected by the medium nitrogen deficiency than phosphate deficiency

**7. *Selenastrum sp.* (Figure 7)**

The survival rate was reduced in media deficient in P and N. It was clear media deficient in N affected the survival rate more than P.

**8. *Chlorococcus sp.* (Figure 8)**

The survival rate was not affected by changing the medium nutrient concentrations. However at concentration 0.15% the depletion of N from the growth medium affected the survival rate than the other media

**Comparison of the chemicals in terms of medium conditions:**

Comparison of the chemicals (Figures 9,10,11) in terms of the response of the green species to Carbon tetrachloride, Chloroform, Trichloroethylene and Tetrachloroethylene under growth conditions

The survival rate of all species was reduced in all treatments especially in media deficient in nitrogen

In case of green algal species it should be concluded:  
N deficient media was effective in reducing survival rate

## **B. Diatoms**

### **1. *Cyclotella* sp. (Figure 12)**

The survival rate of the alga was not affected by P and N deficiency

### **2. *Nitzschia pusilla* sp. (Figure 13)**

The growth rate was affected in media deficient in N specially treated with chloroform and carbon tetrachloride

### **3. *Navicula saprophila* sp. (Figure 14)**

The species was not affected by depleting the media from Si or N

### **4. *Nitzschia dissipata* sp. (Figure 15)**

The survival rate of the alga was affected by Si and N deficiency in the growth medium

### **5. *Thalassiosira weissflogii* sp. (Figure 16)**

The survival rate of the alga was lowered in media deficient in Si and N

### **6. *Skeletonema costatum* sp. (Figure 17)**

The survival rate of the alga was affected by Si and N deficiency

### **7. *Amphiprora hyalina* sp. (Figure 18)**

The survival rate of the alga was not affected by changing the nutrient of the medium.

### **8. *Thalassiosira pseudonana* sp. (Figure 19)**

The survival rate of the alga was reduced in media deficient in N

### **9. *Cylindrotheca* sp. (Figure 20)**

The survival rate of the alga was reduced in media deficient in Si and N

### **10. *Chaetoceros* sp. (Figure 21)**

The survival rate of the alga was not much affected by changing the nutrients of the medium

**11. *Minutocellus* sp. (Figure 22)**

The survival rate of the alga was not much affected by changing the nutrients of the medium

**Comparison of the chemicals in terms of growth conditions:**

comparison of the chemicals (Figures 23, 24,25,26) in terms of the response of the diatoms species to Carbon tetrachloride, Chloroform, Tetrachloroethylene and Trichloroethylene under growth conditions.

**Carbon Tetrachloride: (Figure 23)**

*Nitzschia dissipata*, *Skeletonema* and *Minutocellus* were more sensitive to Si and N deficiency than the other species and as a result their survival rate was lowered

**Chloroform: (Figure 24)**

When species treated with the chemical in growth media deficient in Si and N, the survival rate of *Nitzschia dissipata* and *Thalassiosira weissflogii* were lower than the other species.

**Tetrachloroethylene: (Figure 25)**

*Thalassiosira weissflogii*, *skeletonema*, *Amphiprora*, *Thalassiosira pseudonana*, *Cylindrotheca*, and *Chaetoceros*: The mentioned diatom species were sensitive to nutrient deficiency.

**Trichloroethylene: (Figure 26)**

*Nitzschia dissipata* and *Thalassiosira weissflogii* although they were tolerant of the solvents in complete media, they were sensitive and their survival rate was reduced in the media depleted from Si and N.

## **II. Salinity:**

**DIATOMS:** (Figure 27, 28, 29, 30)

The salinity did not change the survival rate of the diatoms in response to salinity however, they responded at different salinities sometimes even higher than lower salinity. The reason for that is that some of the diatoms grow at different salinities some of them require 18g/L others require 36g/L and others require 54g/L for growth.

## **Conclusion:**

. with respect to changes in the growth composition it was clear in this work that depletion of nutrients nitrogen or phosphate in case of green algal species or silicate in the case of diatoms lowers the percentage of survival of the organism.

. Green algal species were more or less tolerant to changing growth media composition than diatoms.

## **FUTURE PLANS:**

We will continue to investigate the effect of halogenated hydrocarbons on the aquatic organisms in the following experiments:

.The effect of the chemicals will be assayed in growth media complete and deficient in one element (nitrogen as nitrate, phosphorus as phosphate or silican as silicate).

.The response of algal species to the chemicals will be determined in the original medium after being enriched with various sea salt concentrations 15, 25 or 35 ppt (parts per thousands).

.The above experiments will be performed with mixed species.

. The accumulation of the solvents will be determined by the algal species using Gas liquid Chromatography.

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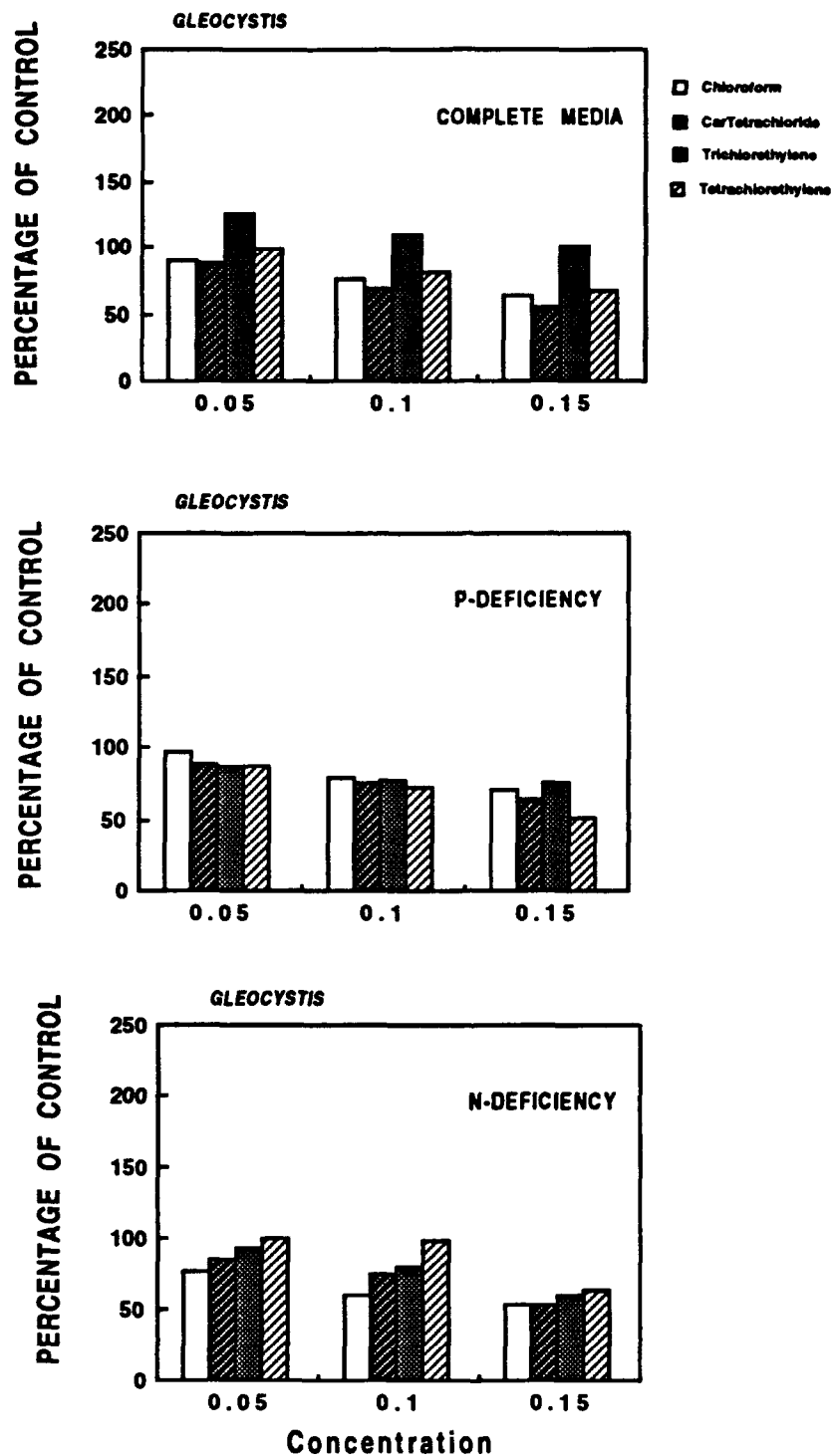


Figure1: Effect of chemicals on growth of green alga *Gleocystis* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%



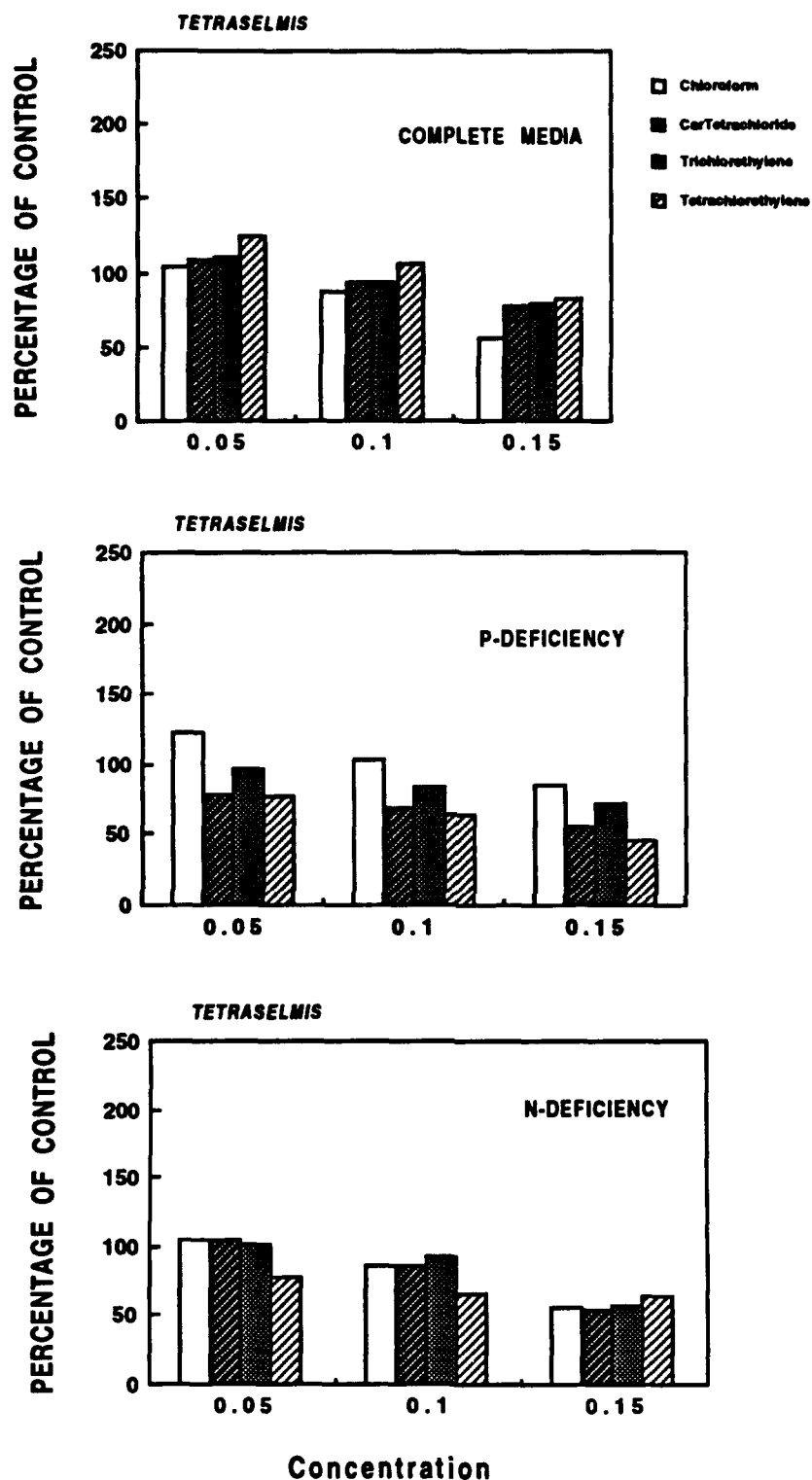


Figure 2: Effect of chemicals on growth of green alga *Tetraselmis sp.* in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

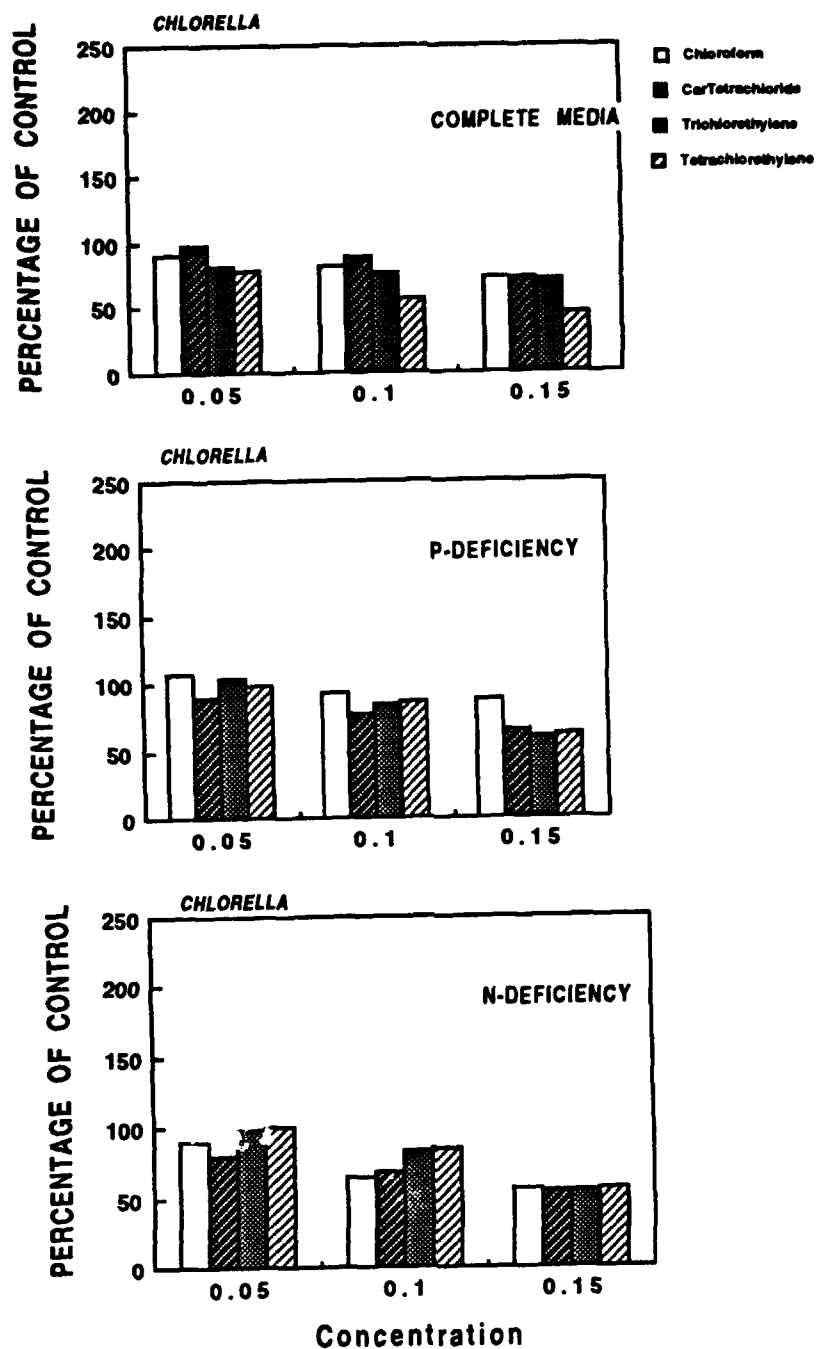


Figure 3: Effect of chemicals on growth of green alga *Chlorella* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

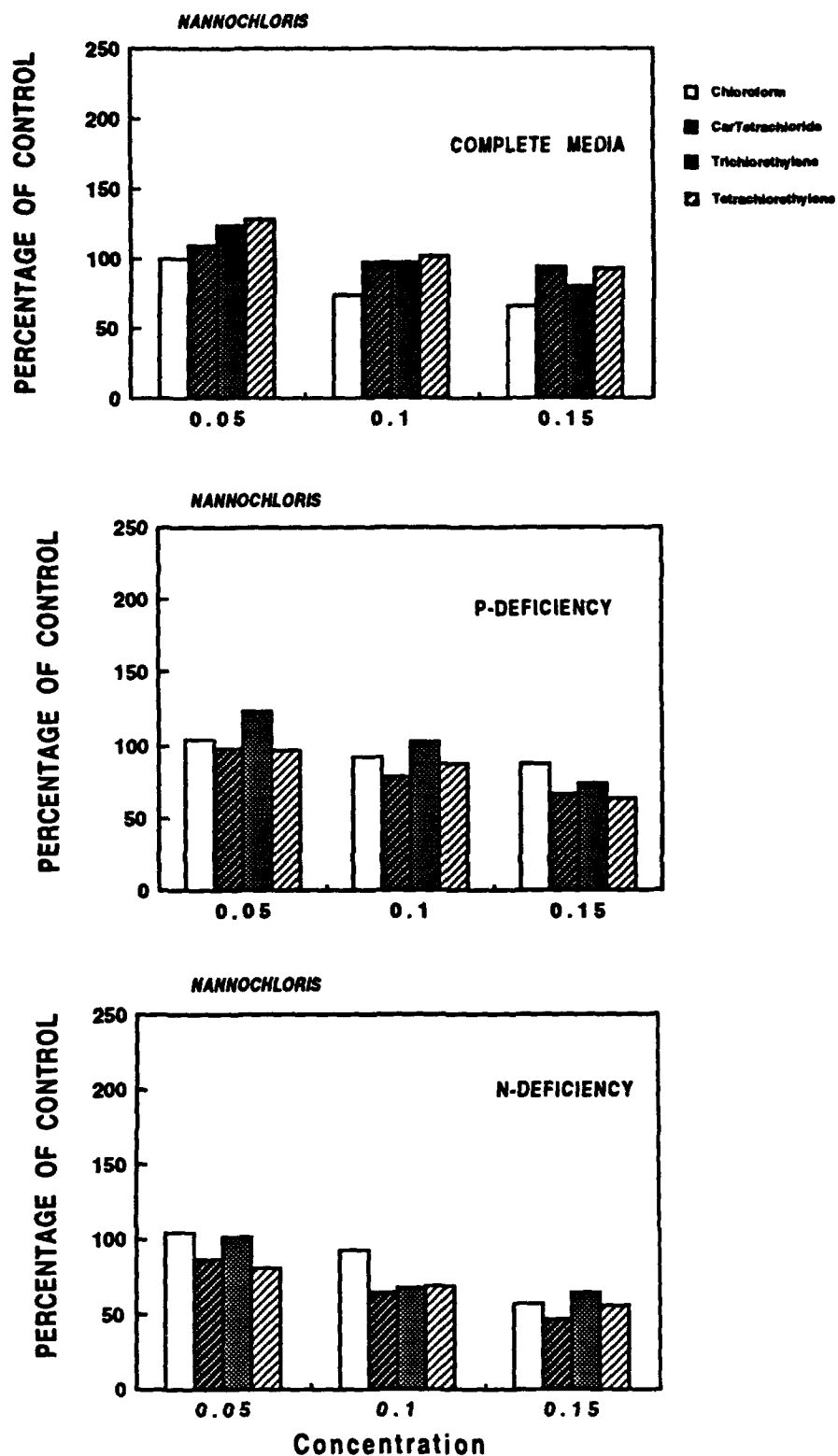


Figure 4: Effect of chemicals on growth of green alga *Nannochloris* 3 sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

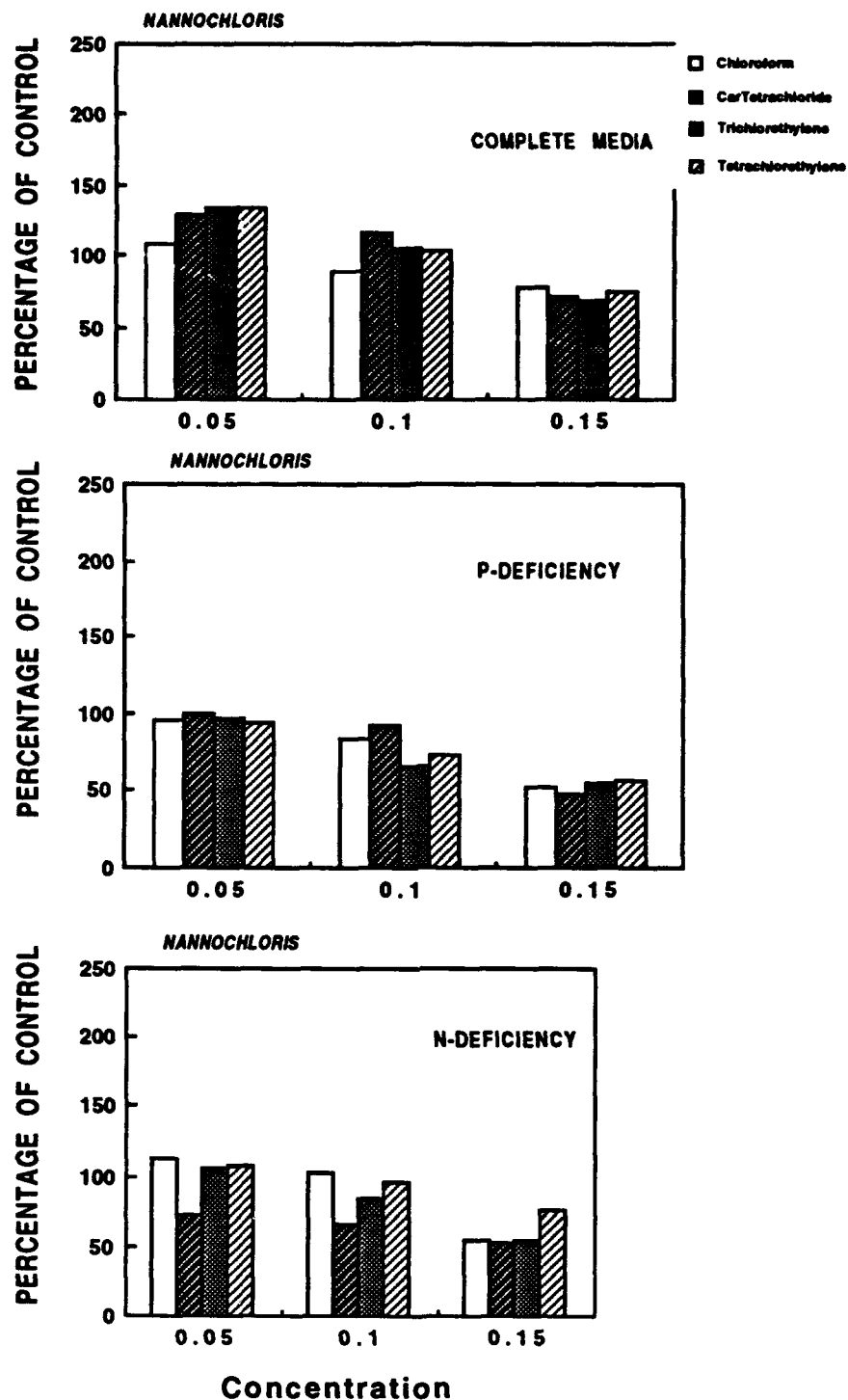


Figure 5: Effect of chemicals on growth of green alga *Nannochloris* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

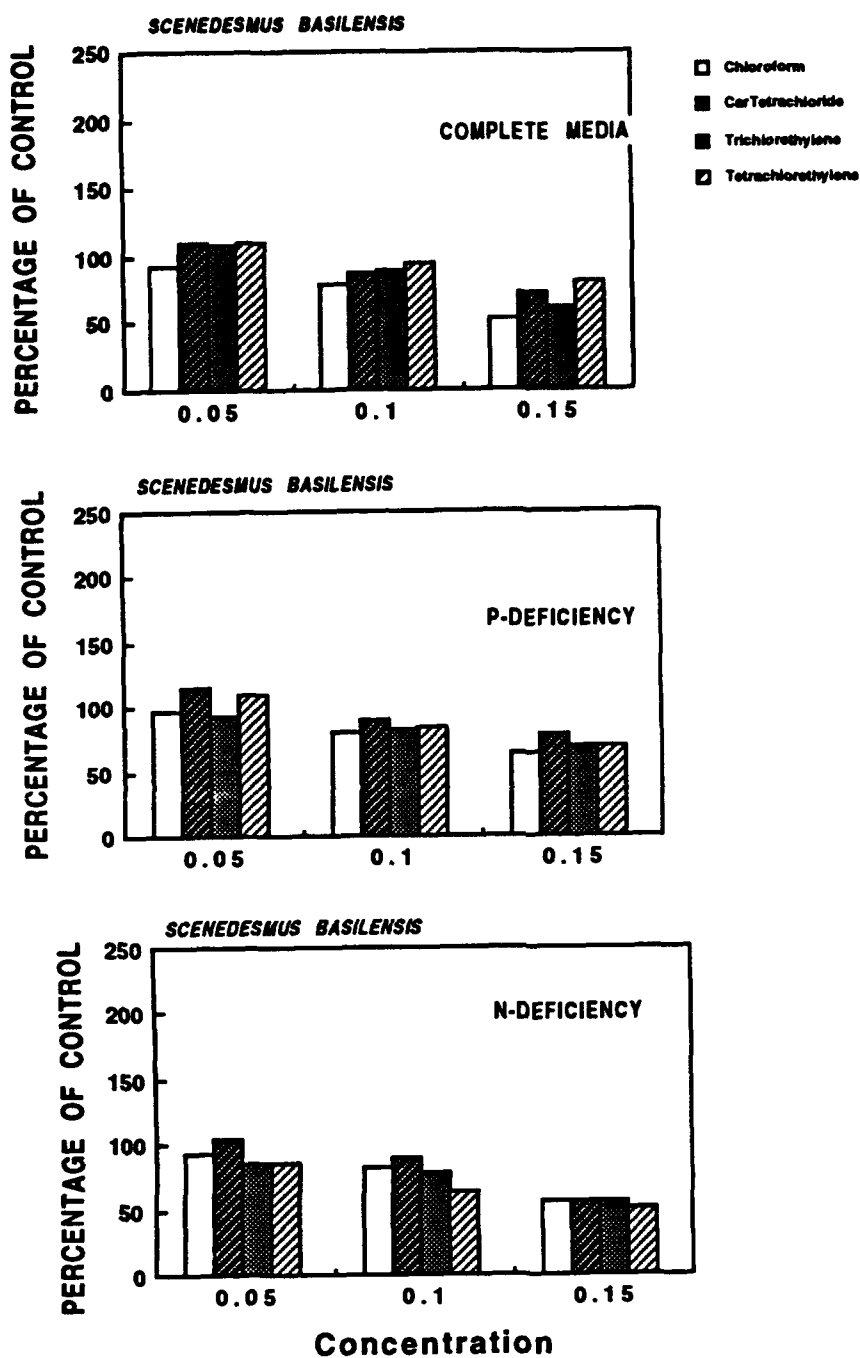


Figure 6: Effect of chemicals on growth of green alga *Scenedesmus* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

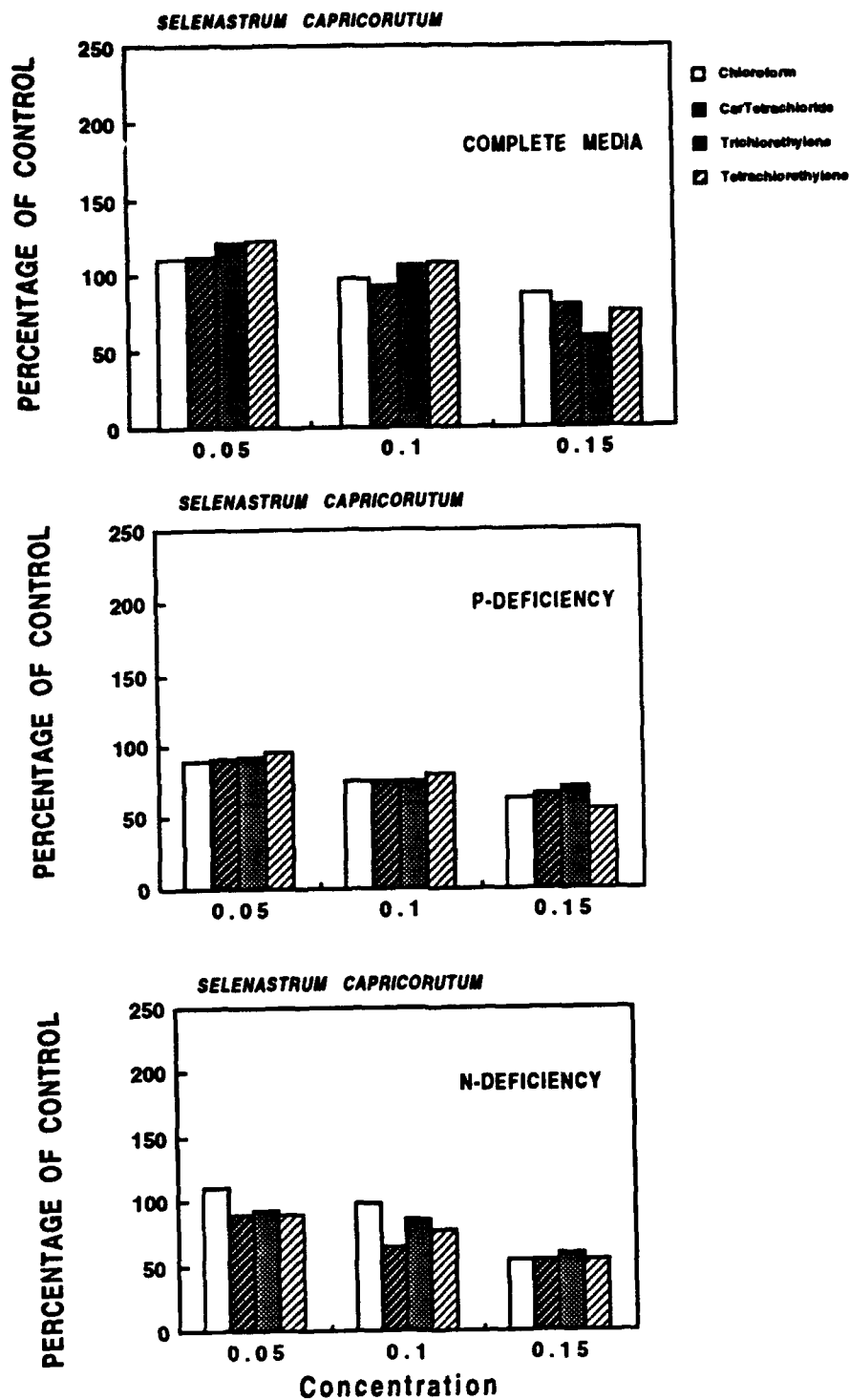


Figure 7: Effect of chemicals on growth of green alga *Selenastrum* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

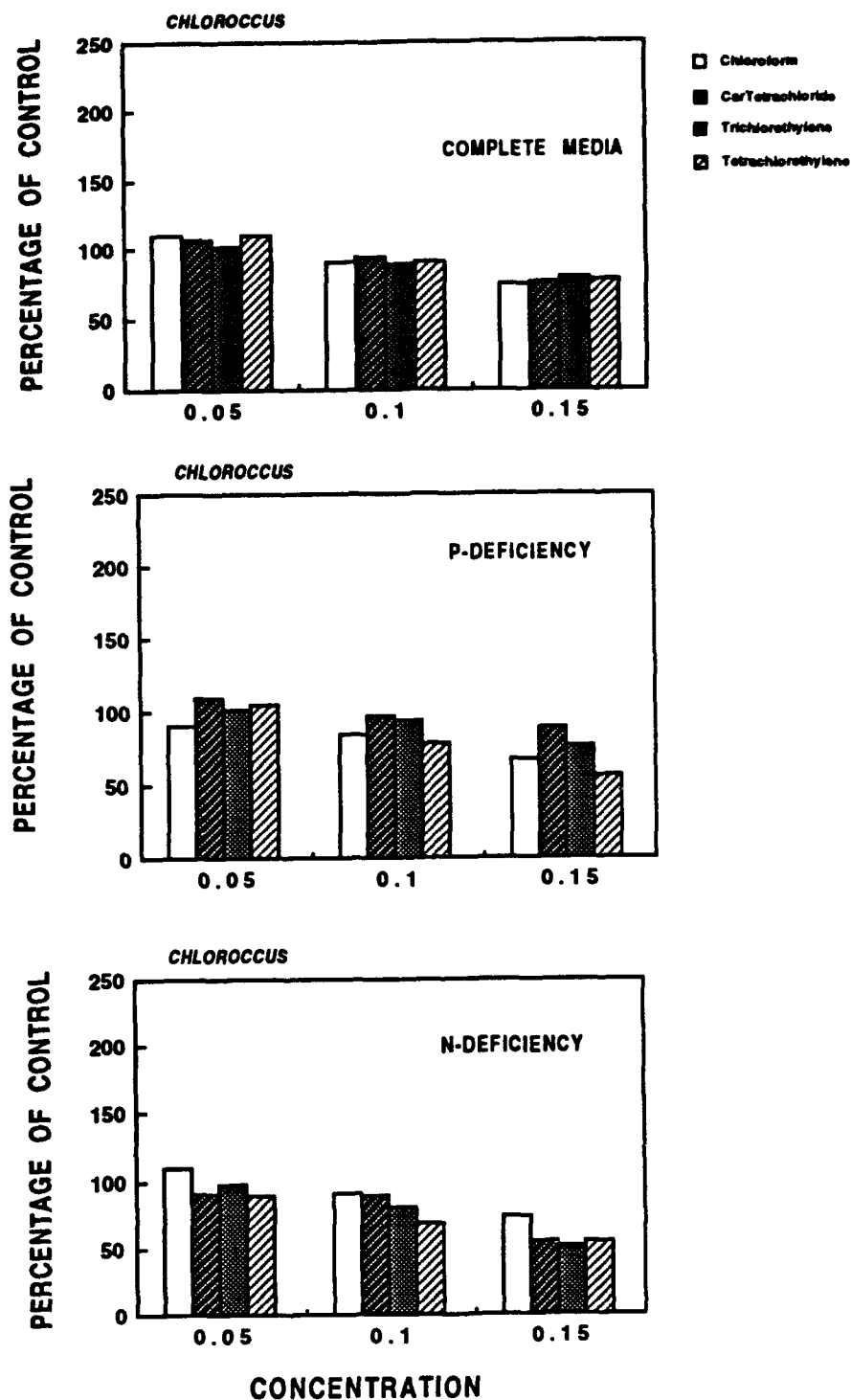


Figure 8: Effect of chemicals on growth of green alga *Chlorococcus* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

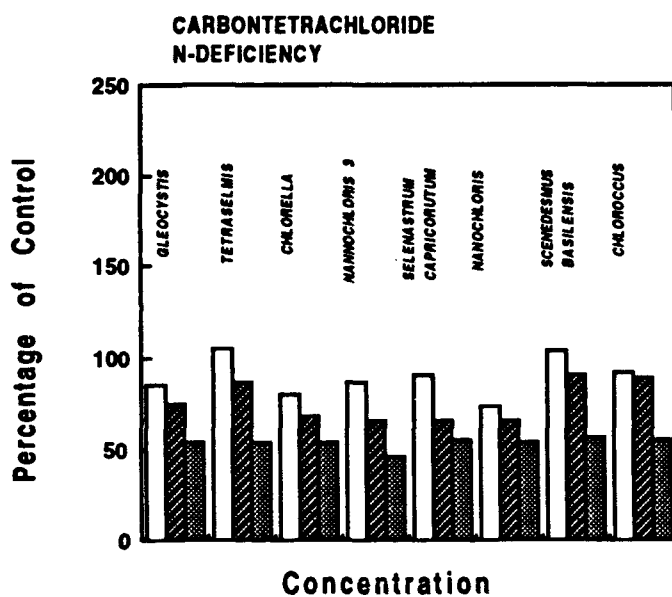
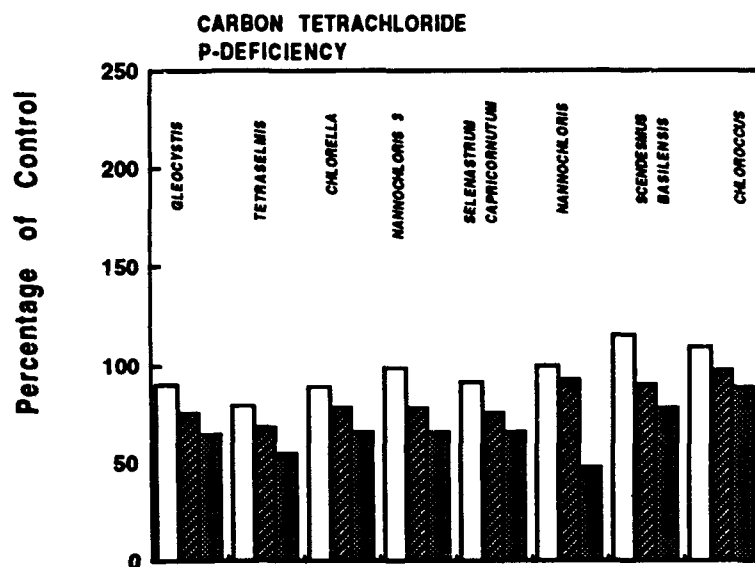
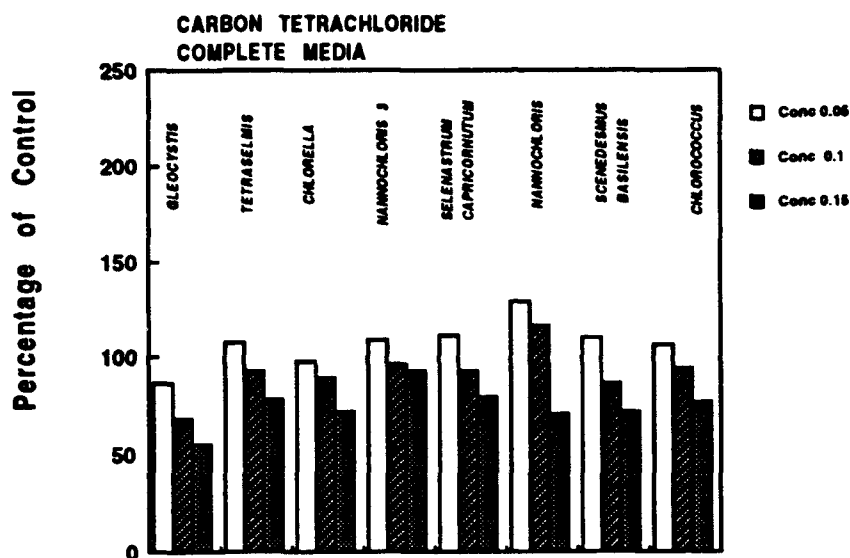


Figure 9: Response of green species to chemicals in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%



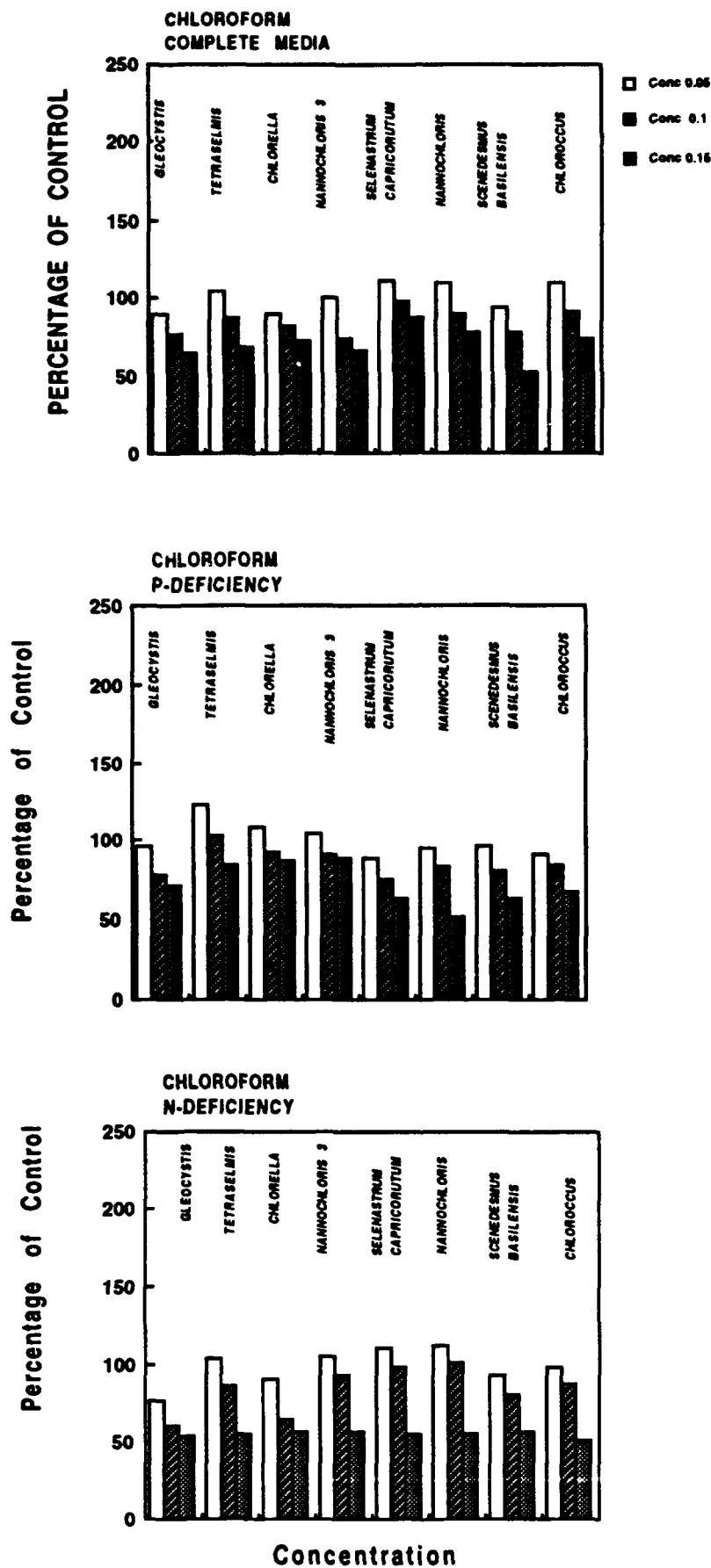


Figure 10: Response of green species to chemicals in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

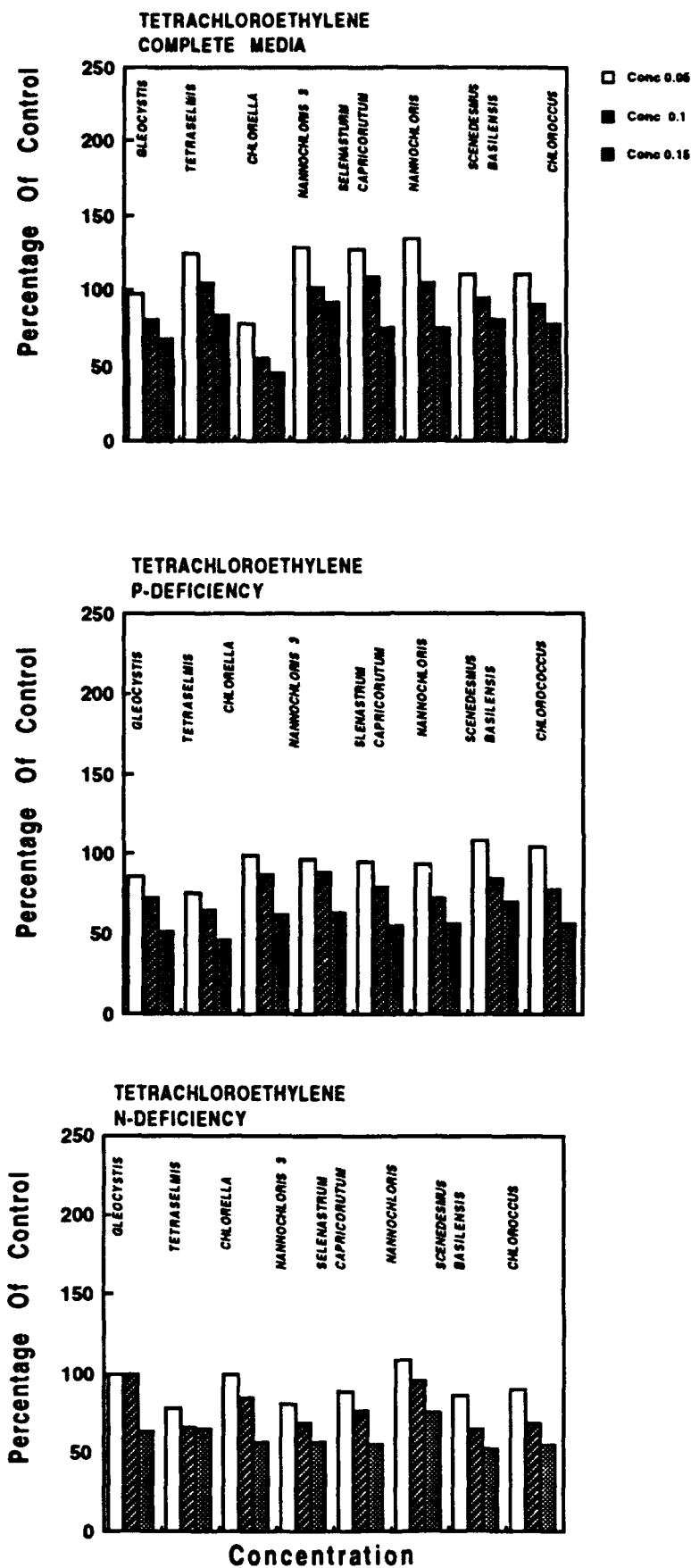


Figure 11: Response of green species to chemicals in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

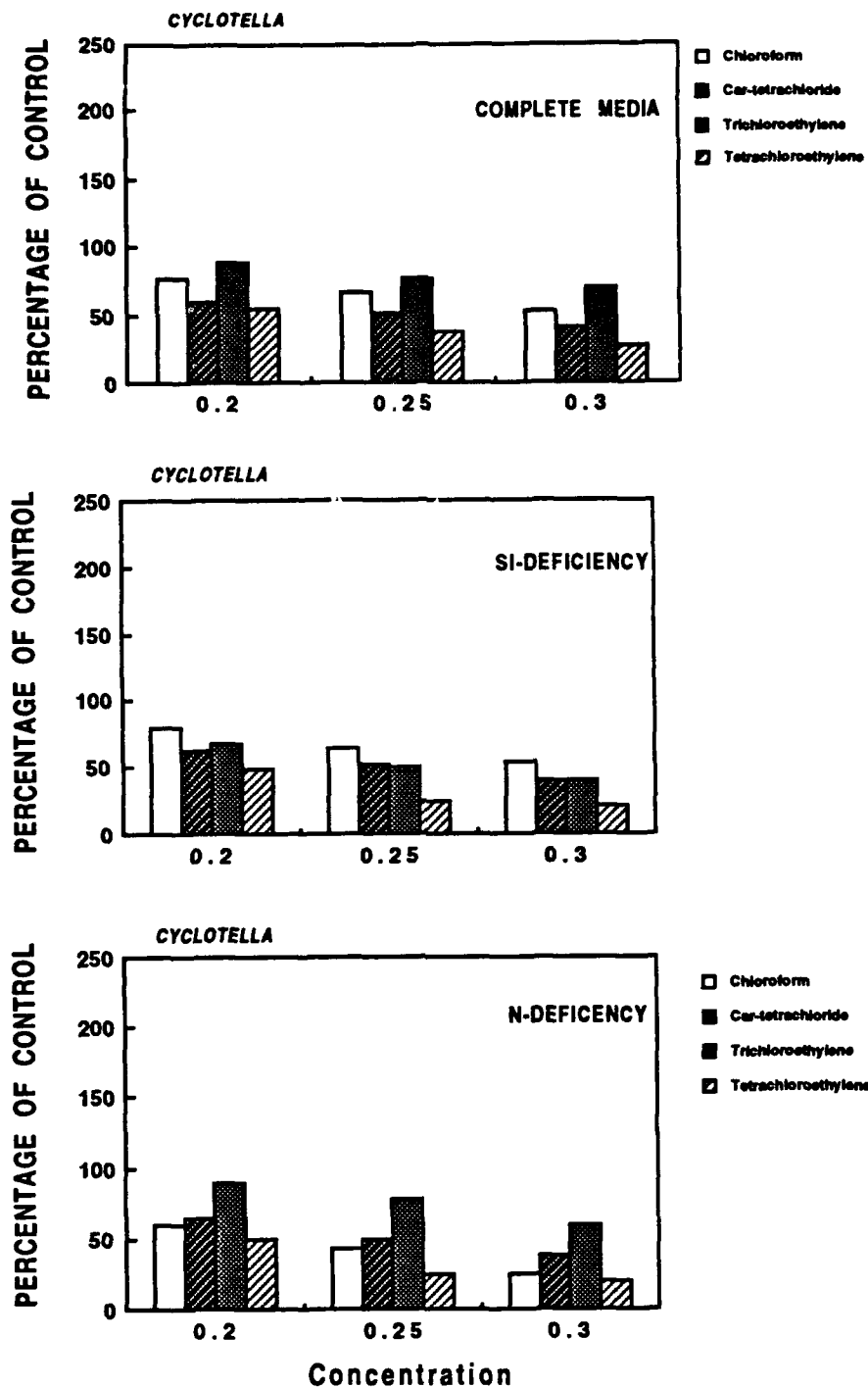


Figure 12: Effect of chemicals on growth of diatom, *cyclotella* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

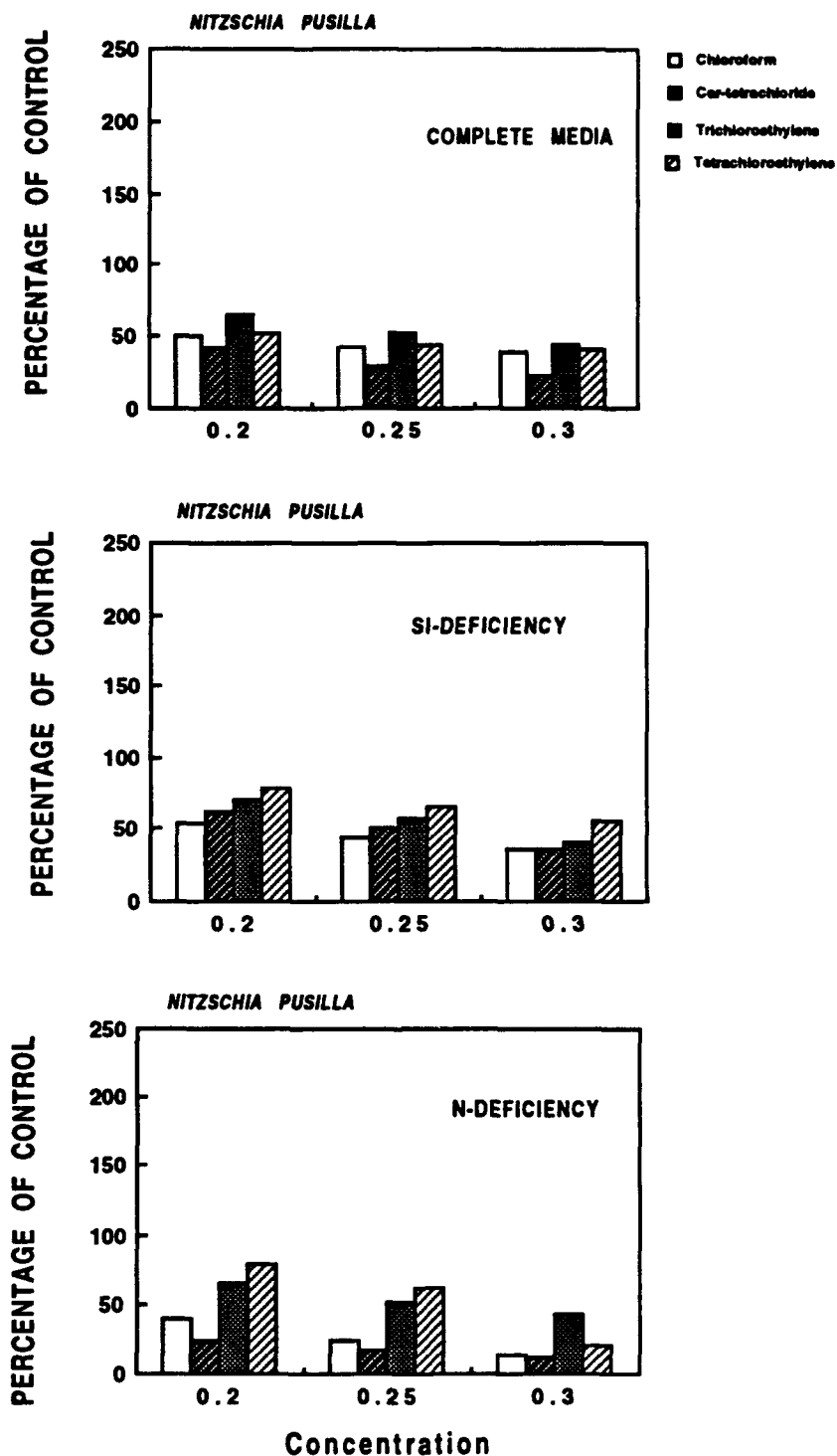


Figure 13: Effect of chemicals on growth of diatom, *Nitzschia pusilla* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

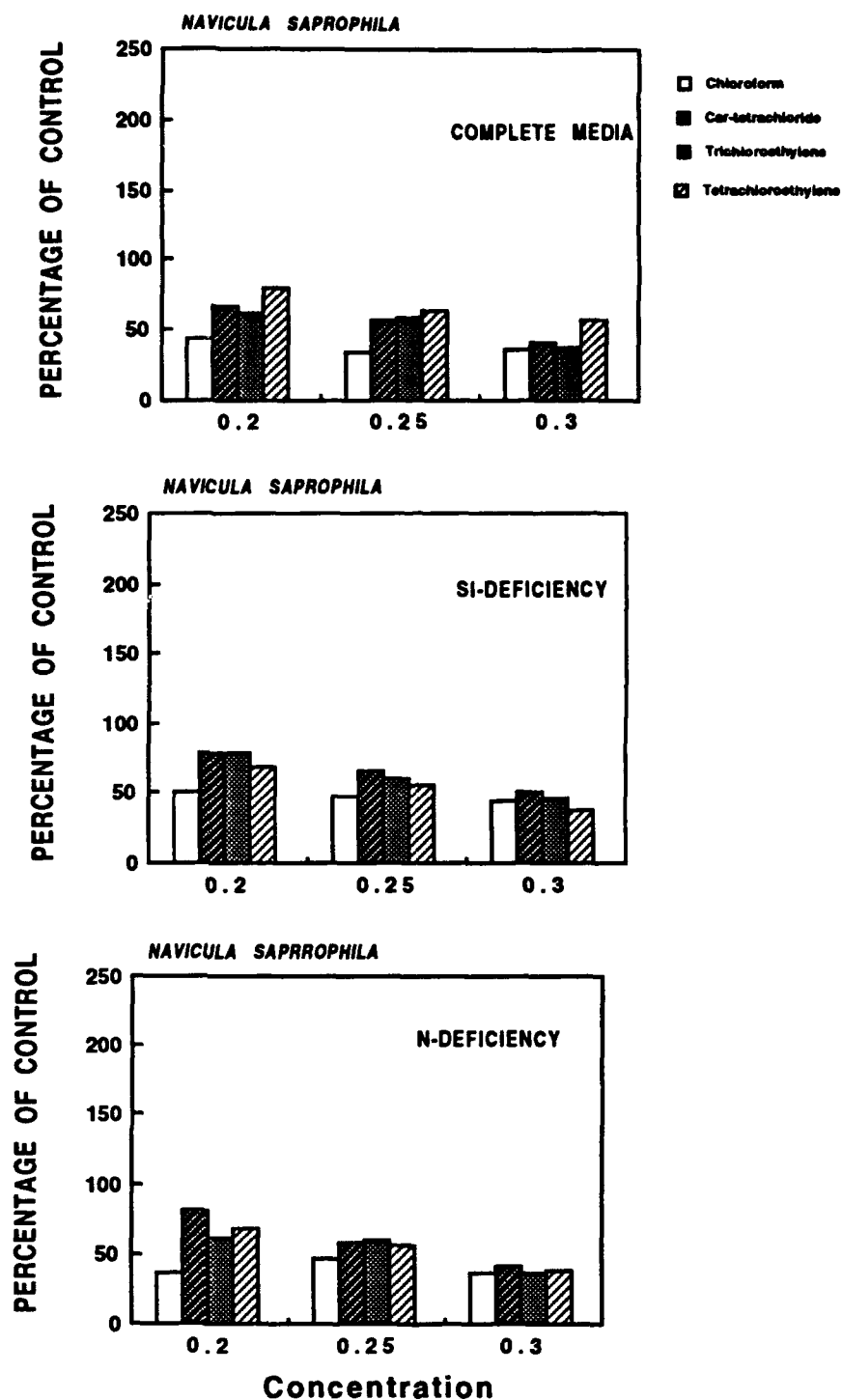


Figure 14: Effect of chemicals on growth of diatom, *Navicula saprophila* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

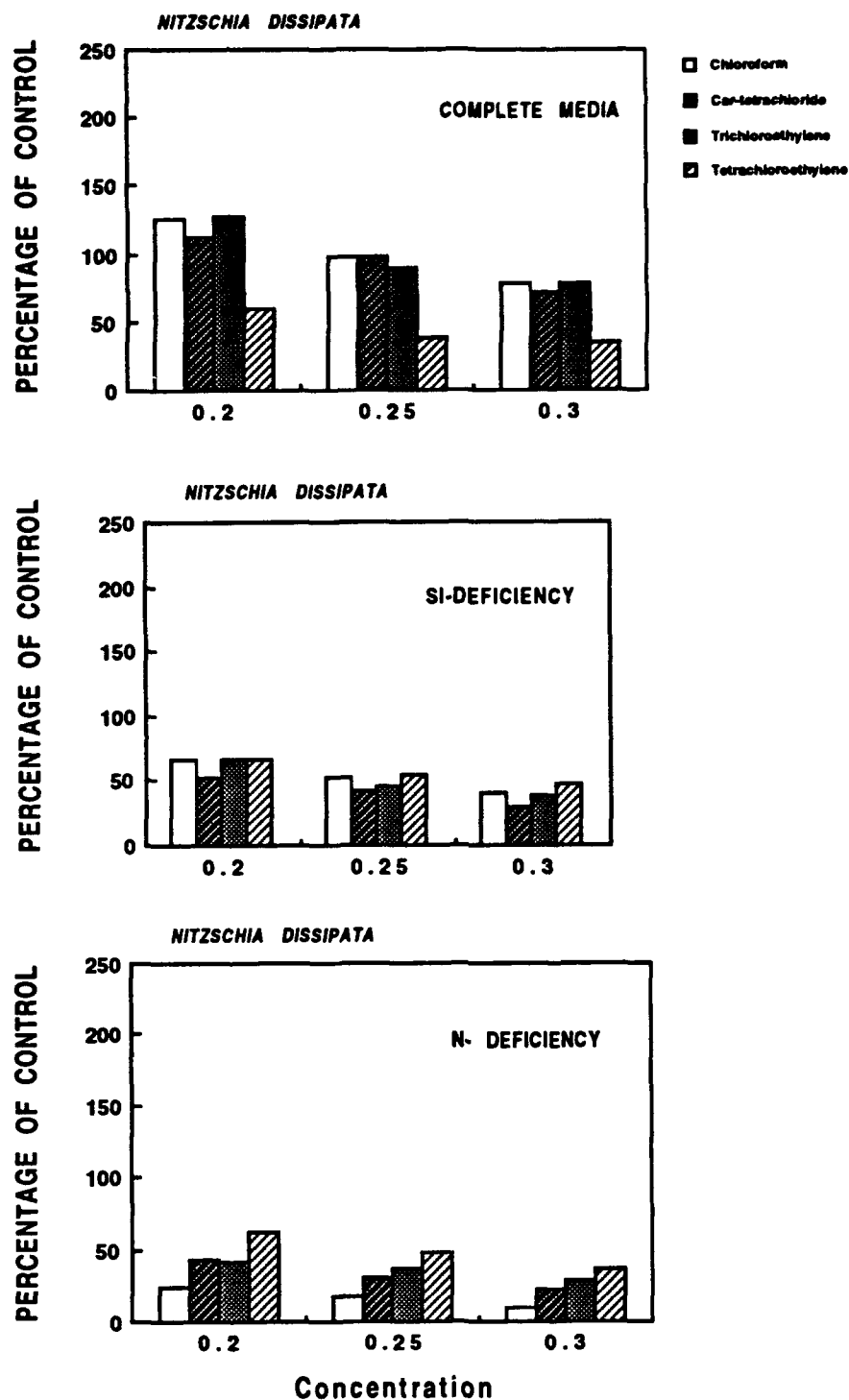


Figure 15: Effect of chemicals on growth of diatom, *Nitzschia dissipata* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

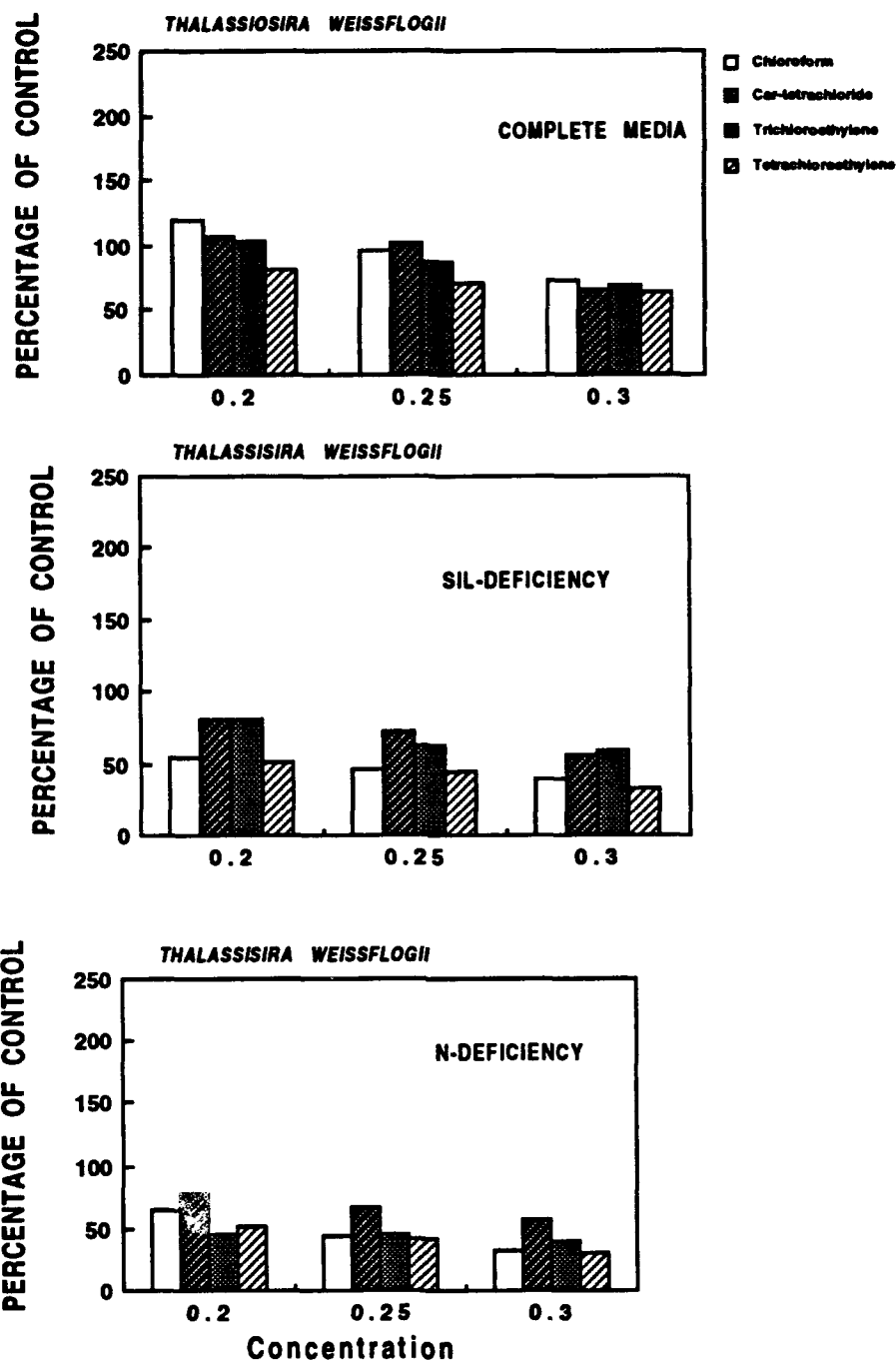


Figure 16: Effect of chemicals on growth of diatom, *Thalassiosira weissflogii* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

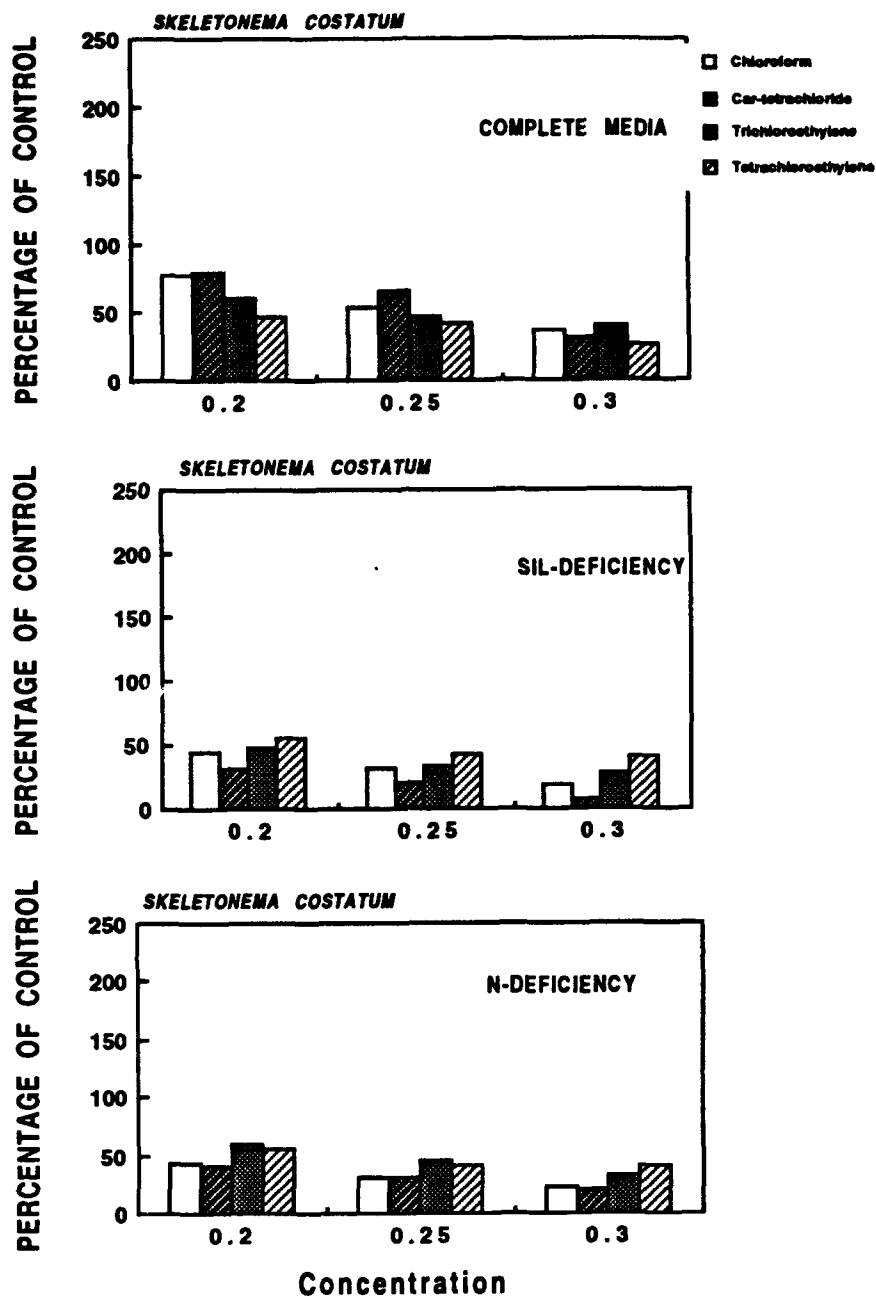


Figure 17: Effect of chemicals on growth of diatom, *Skeleonema costatum* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%



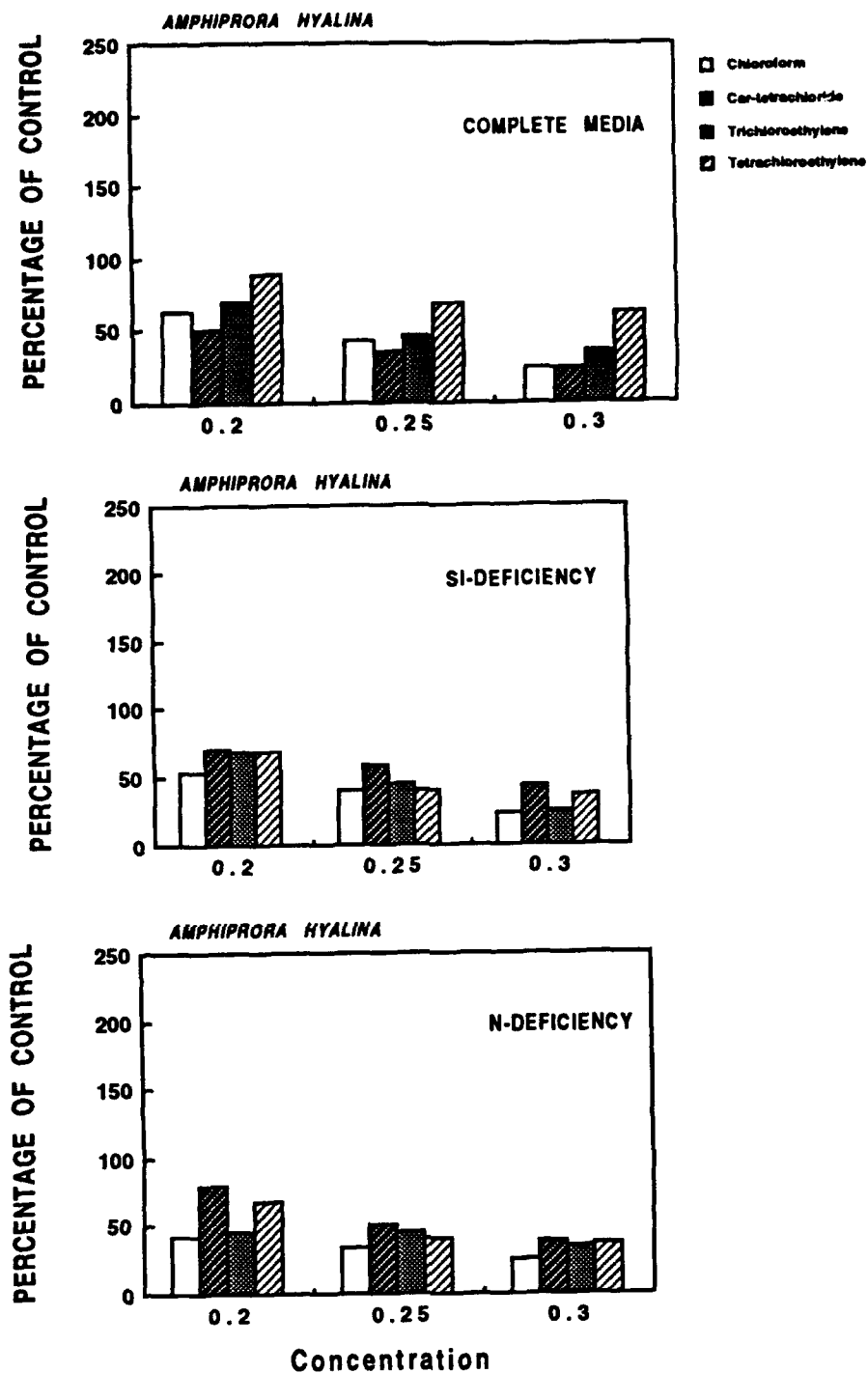


Figure 18: Effect of chemicals on growth of diatom, *Amphiprora hyalina* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

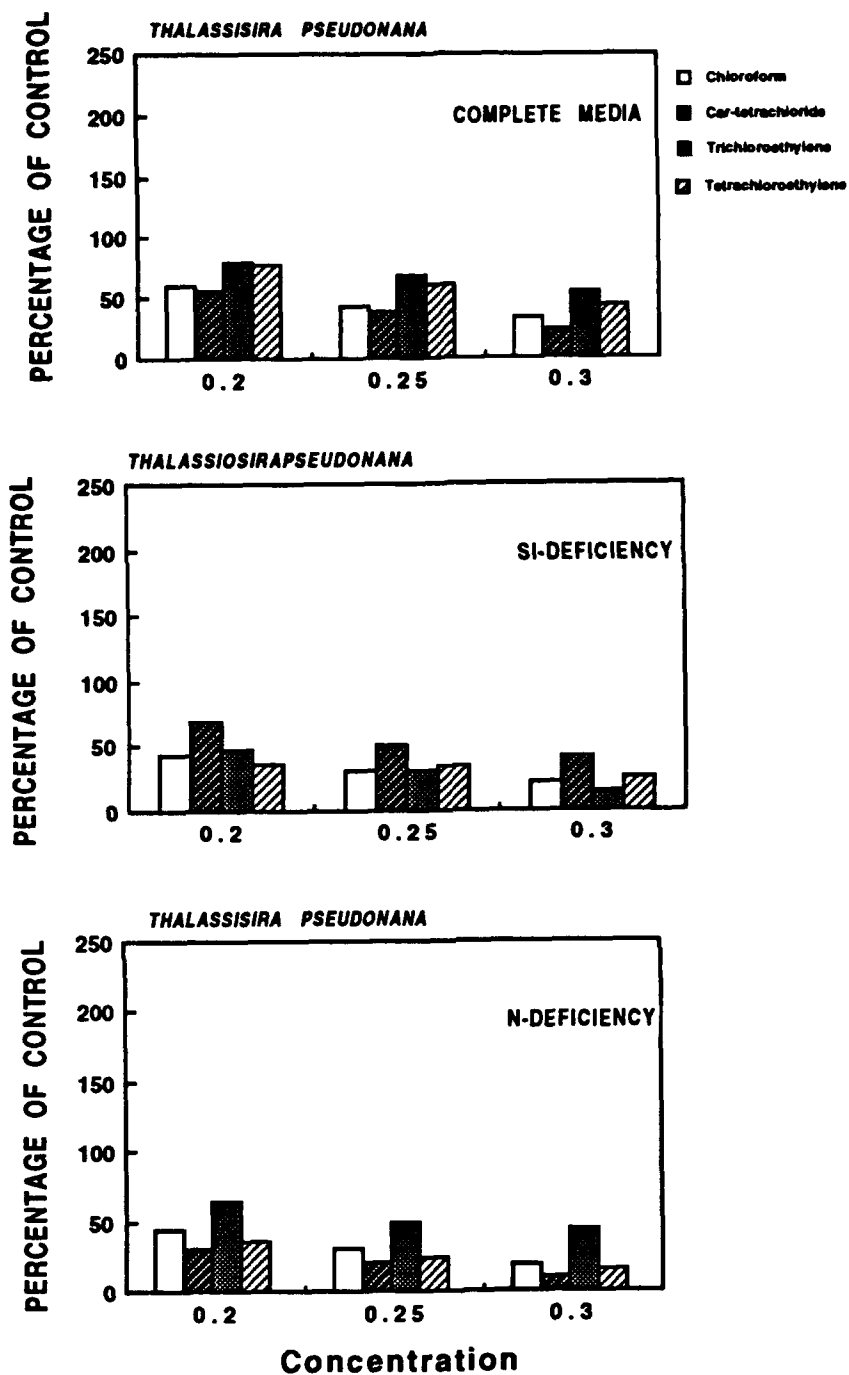


Figure19: Effect of chemicals on growth of diatom, *Thalassiosira pseudonana* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

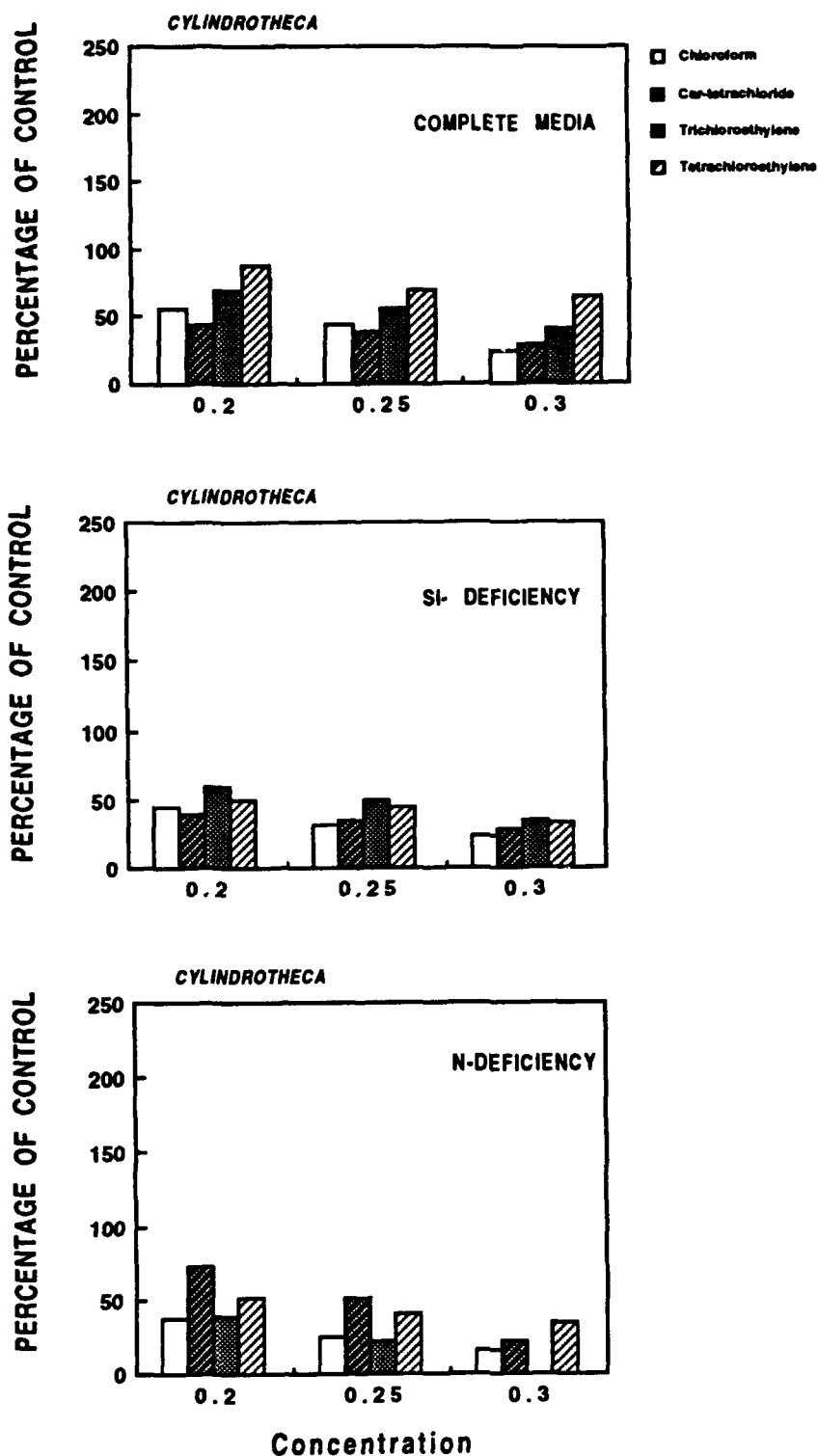


Figure 20: Effect of chemicals on growth of diatom, *Cylindrotheca* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

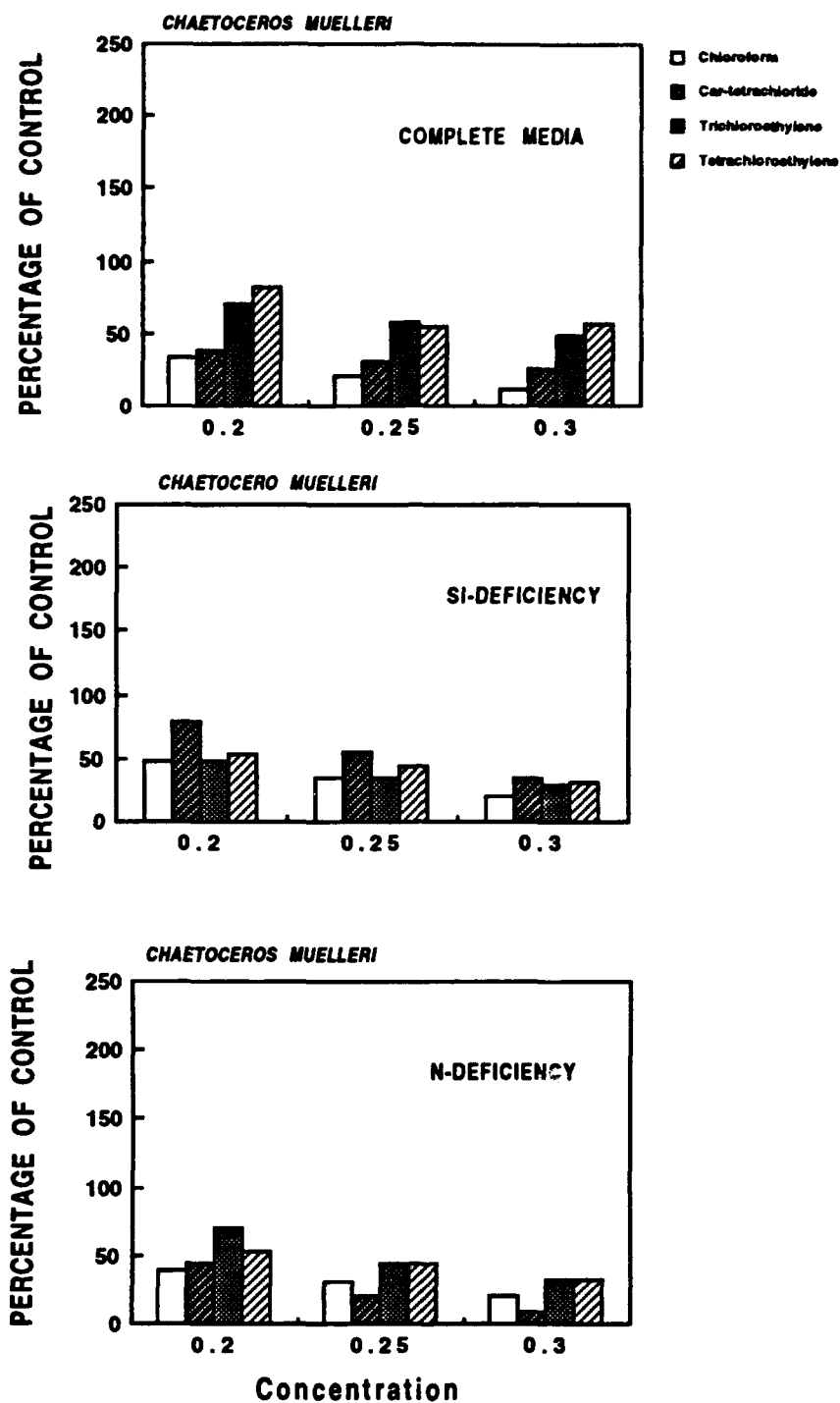


Figure 21: Effect of chemicals on growth of diatom, *Chaetoceros sp.* in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

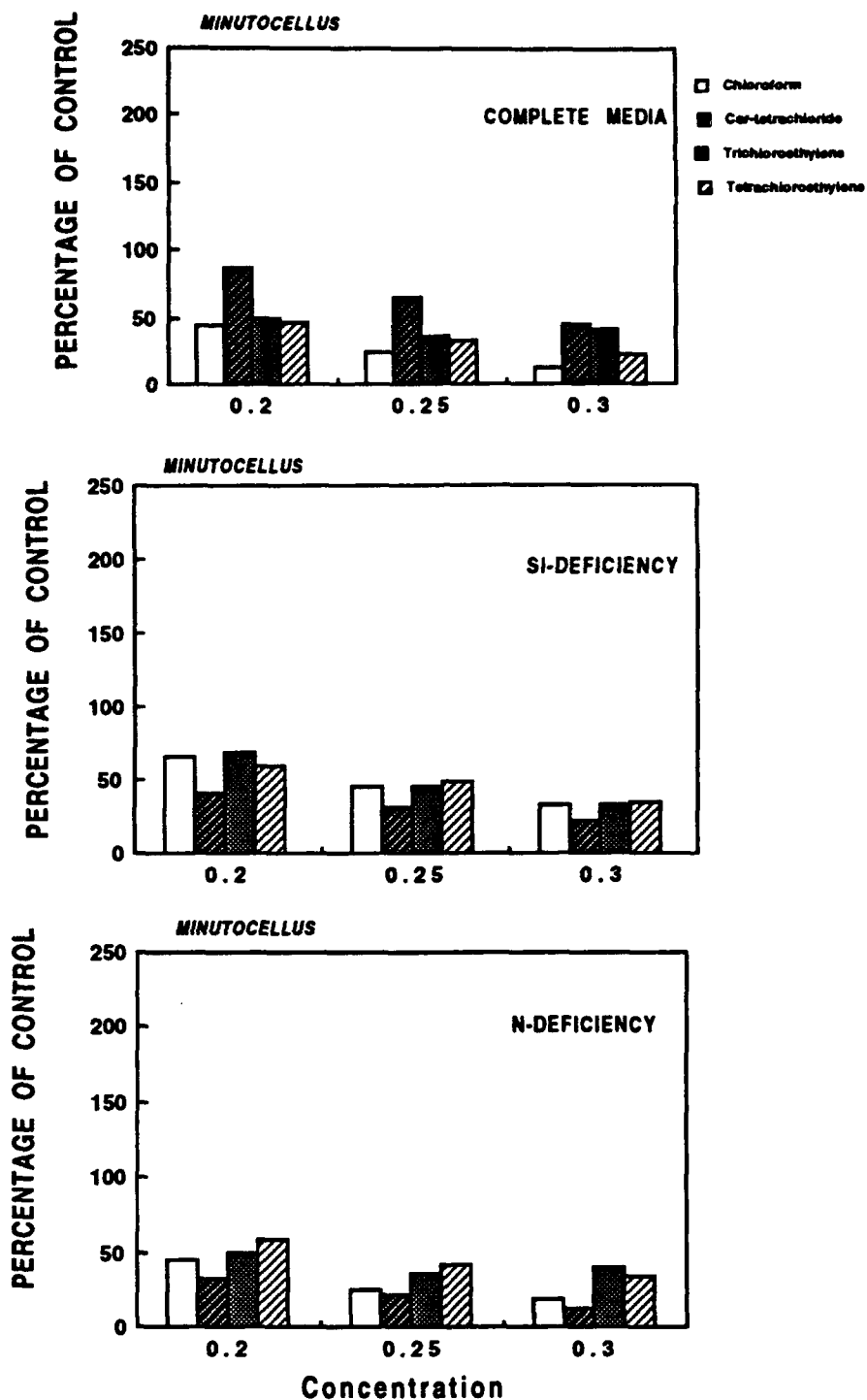


Figure 22: Effect of chemicals on growth of diatom, *Minutocellus* sp. in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

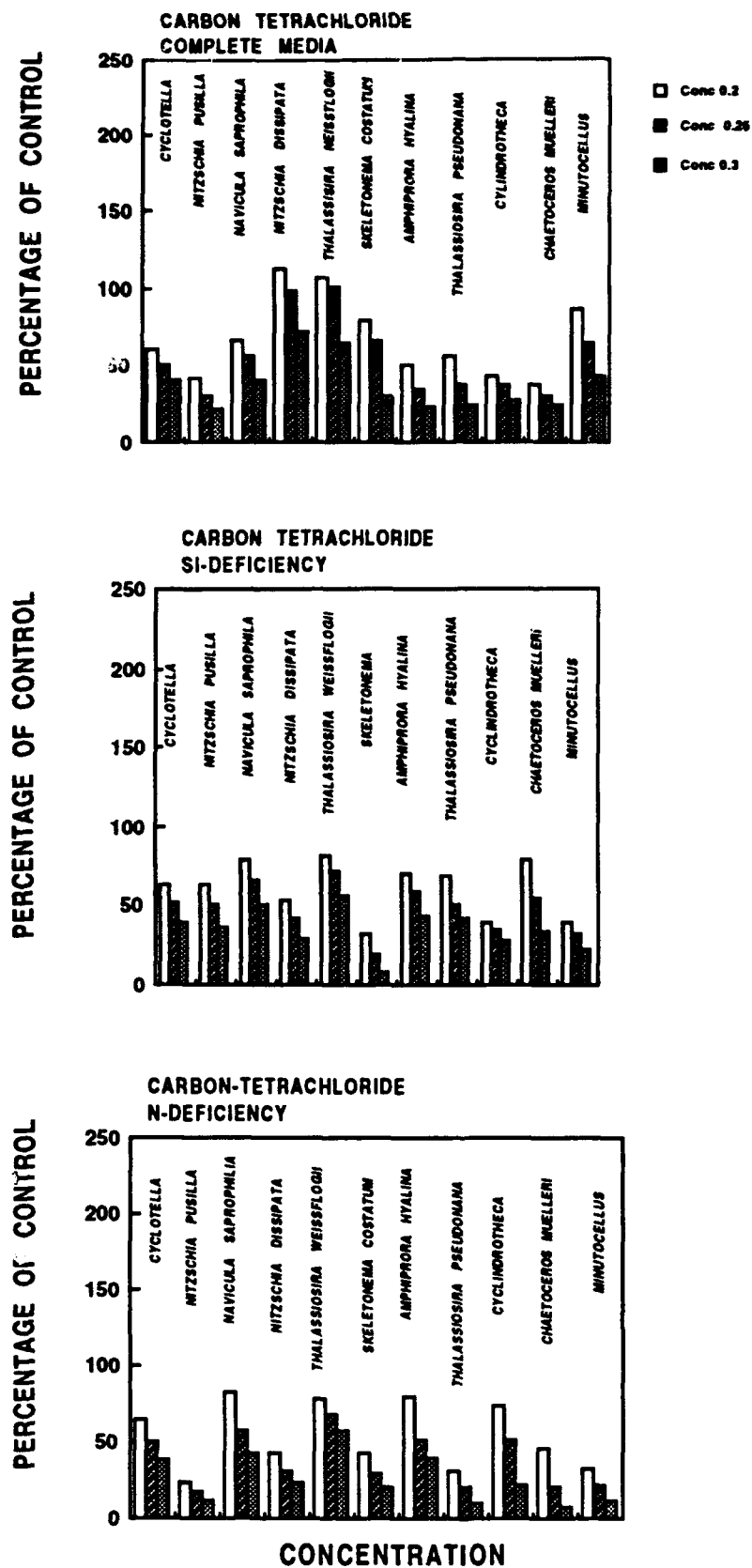


Figure 23: Response of diatom species to chemicals, in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

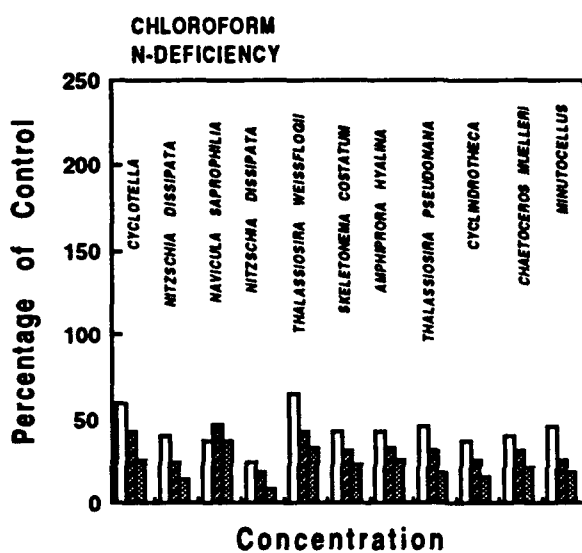
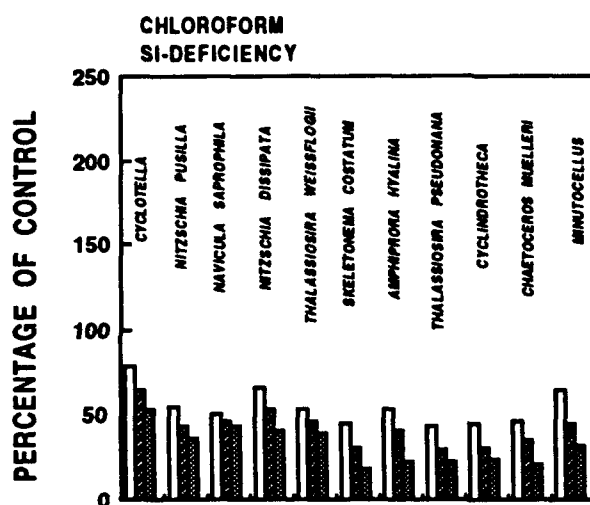
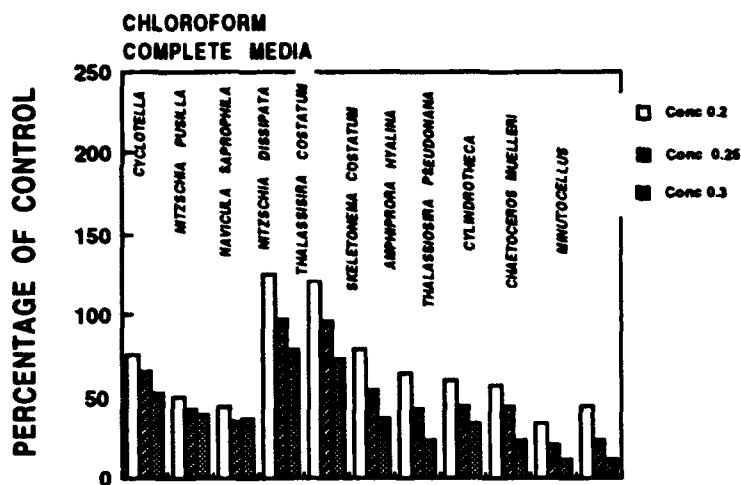


Figure 24: Response of diatom species to chemicals, in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

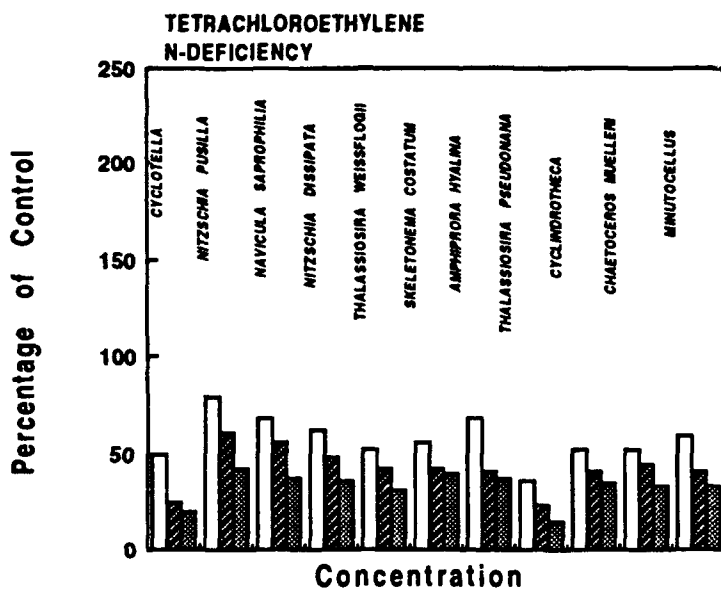
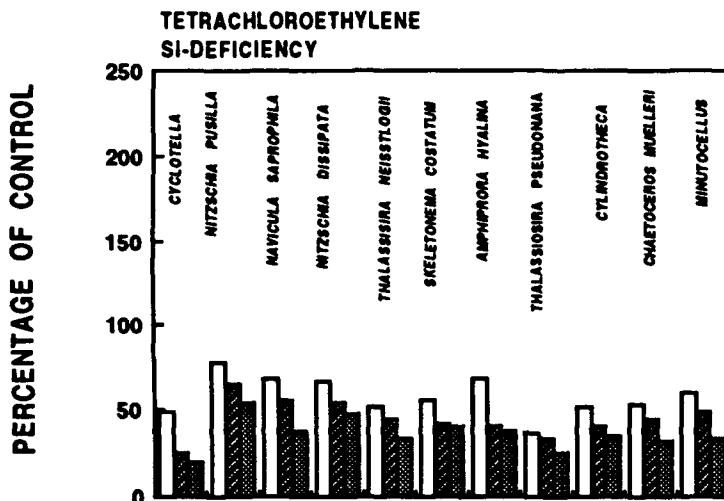
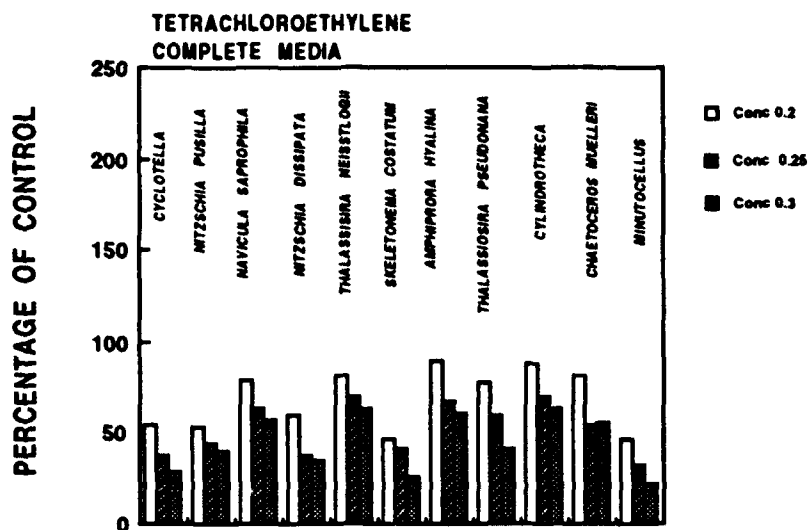


Figure 25: Response of diatom species to chemicals, in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%



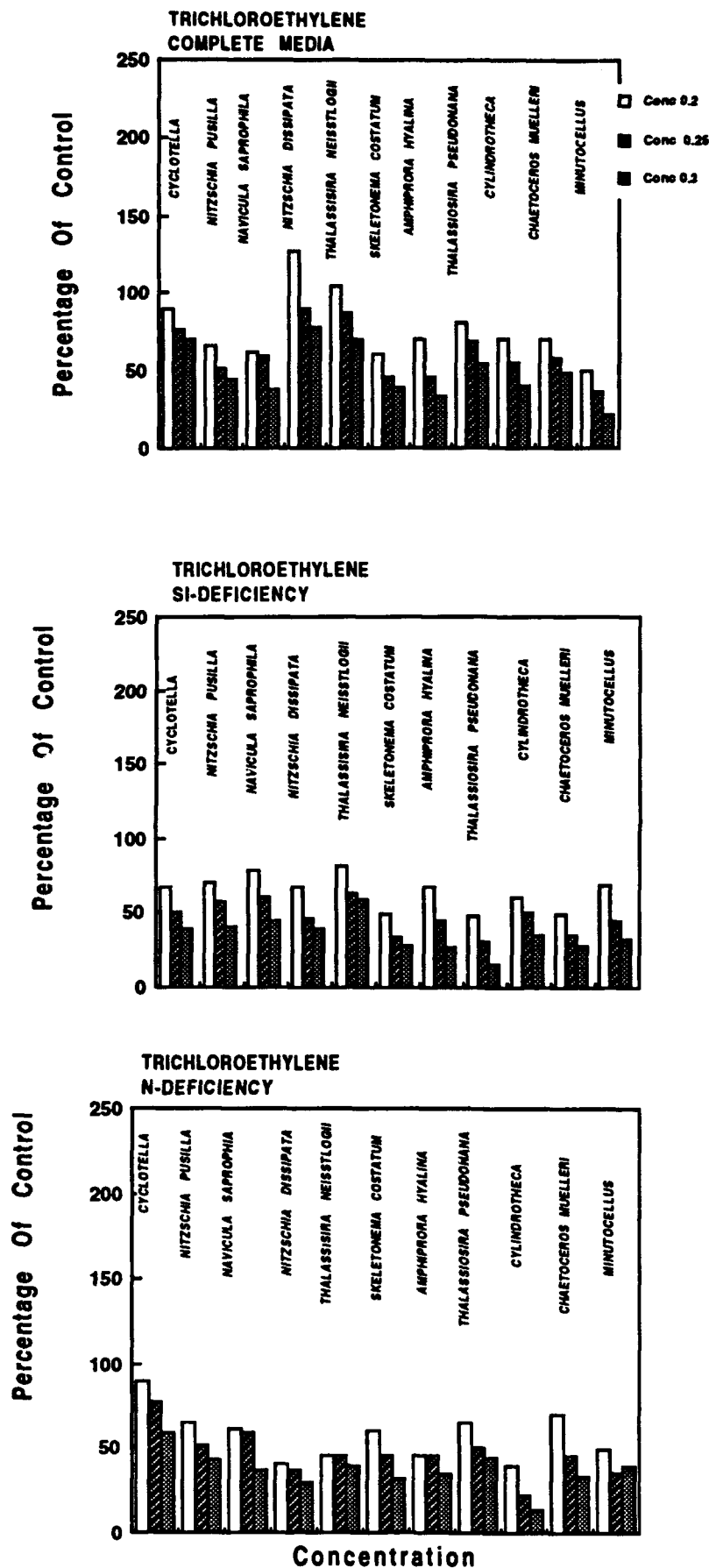


Figure 26: Response of diatom species to chemicals, in complete and deficient media as a percentage of the control. Standard deviation did not exceed 2%

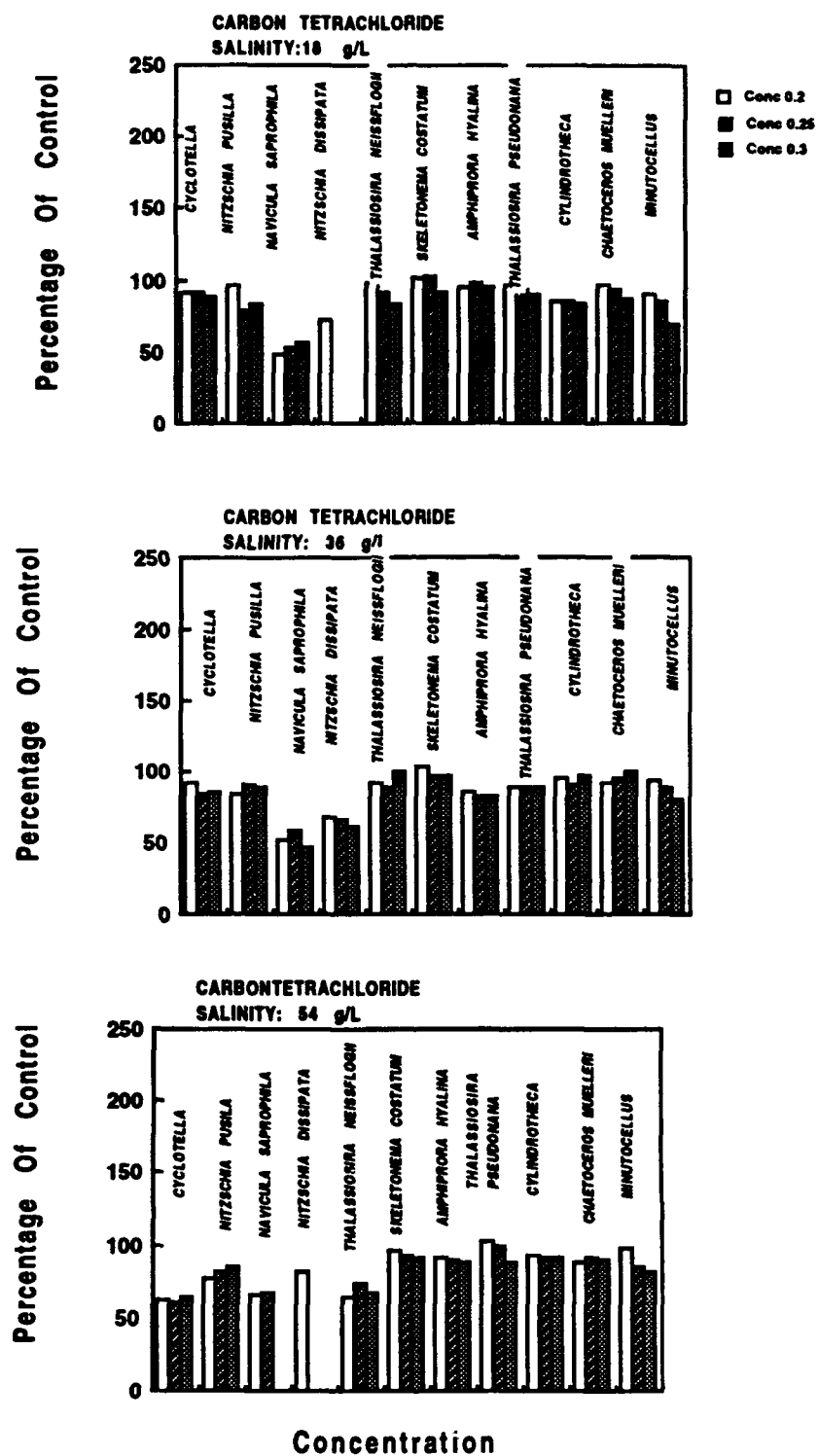


Figure 27: Response of diatom species to chemicals, in media of different salinities as a percentage of the control. Standard deviation did not exceed 2%

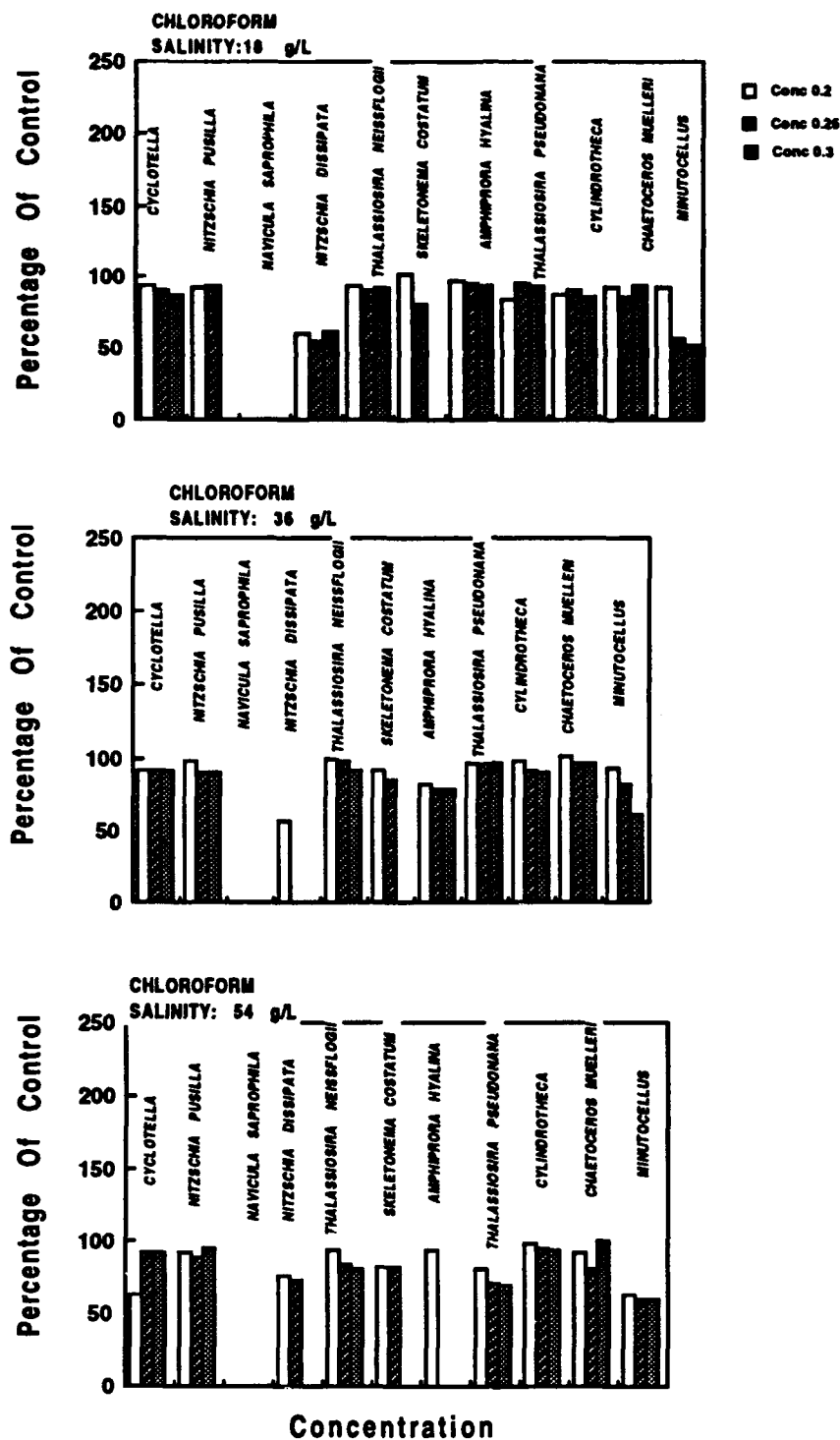


Figure 28: Response of diatom species to chemicals, in media of different salinities as a percentage of the control. Standard deviation did not exceed 2%

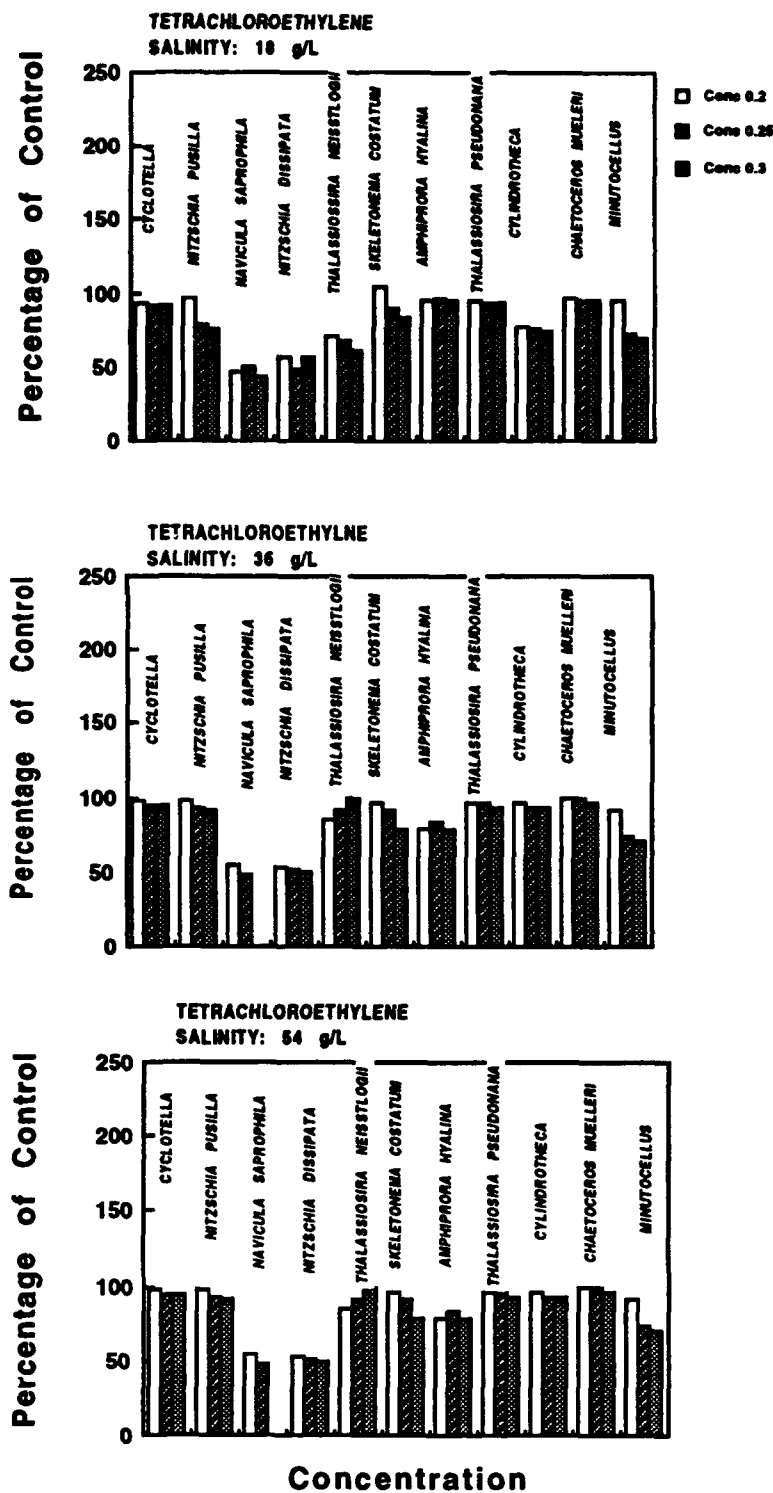


Figure 29: Response of diatom species to chemicals, in media of different salinities as a percentage of the control. Standard deviation did not exceed 2%

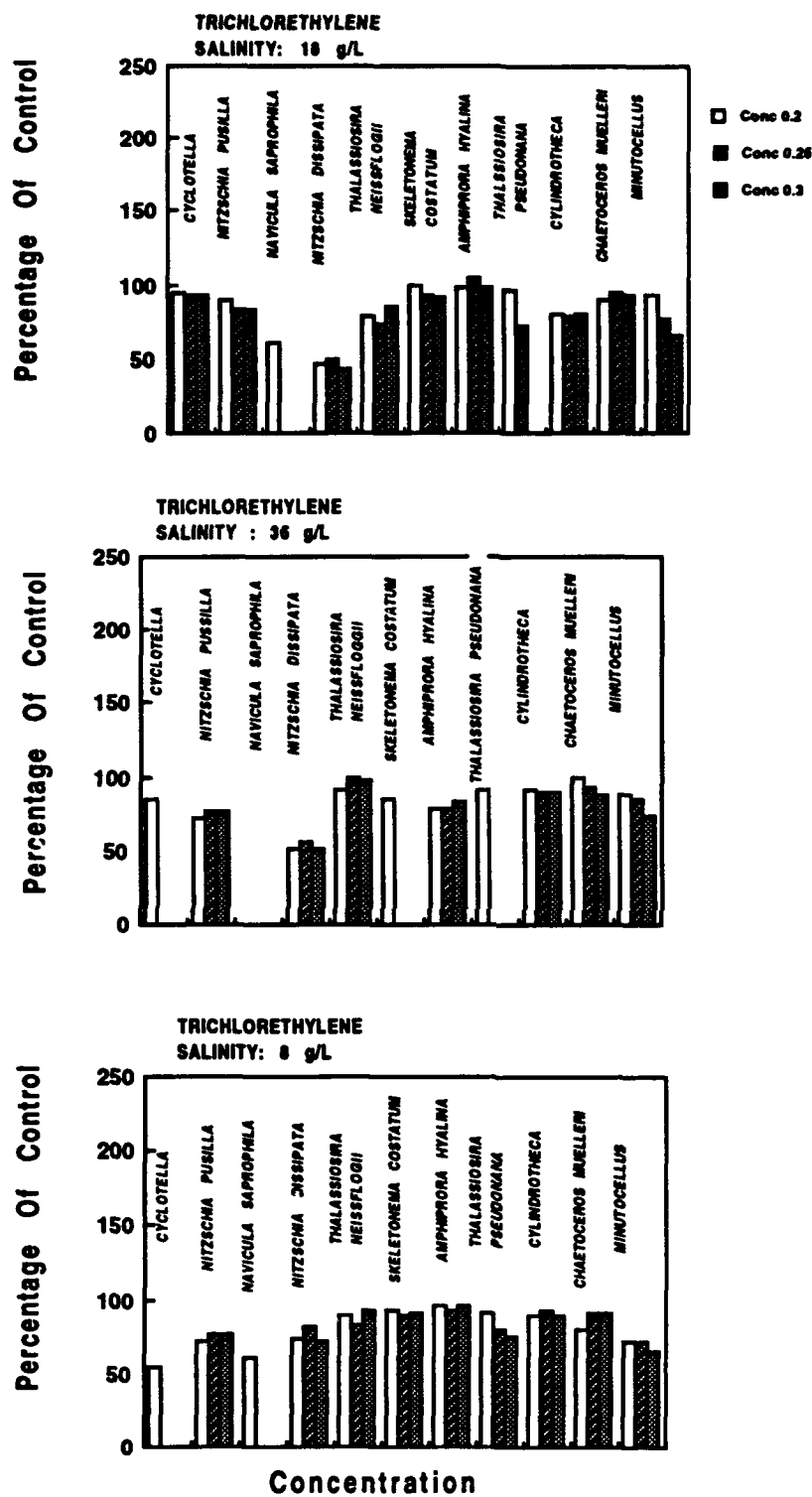


Figure 30: Response of diatom species to chemicals, in media of different salinities as a percentage of the control. Standard deviation did not exceed 2%

## **PERSONNEL**

The following personnel have been involved in this project:

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